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S P E C I F I C A T I O N

COMPOUNDS HAVING SELECTIVE ACTIVITY FOR RETINOID X RECEPTORS, AND MEANS FOR MODULATION OF PROCESSES MEDIATED BY RETINOID X RECEPTORS

RELATED APPLICATIONS

This application is a continuation-in-part of the application Serial No. 08/052,051 filed on April 21, 1993, which is a continuation-in-part of the application Serial No. 08/027,747 filed on March 5, 1993, which is a continuation-in-part of application Serial No. 08/003,223 filed on January 11, 1993, which is a continuation-in-part of application Serial No. 944,783 filed on September 11, 1992, which is a continuation-in-part of application Serial No. 872,707 filed April 22, 1992, whose entire disclosures are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to intracellular receptors and ligands therefor. More specifically, this invention relates to compounds having selective activity for specific retinoic acid receptors, and methods for use of such compounds.

BACKGROUND OF THE INVENTION

The vitamin A metabolite retinoic acid has long been recognized to induce a broad spectrum of biological effects. A variety of structural analogues of retinoic acid have been synthesized that also have been found to be bioactive. Some,

such as Retin-A® (registered trademark of Johnson & Johnson) and Accutane® (registered trademark of Hoffmann-LaRoche), have found utility as therapeutic agents for the treatment of various pathological conditions. Metabolites of vitamin A and their

5   synthetic analogues are collectively herein called "retinoids".

Synthetic retinoids have been found to mimic many of the pharmacological actions of retinoic acid. However, the broad spectrum of pharmacological actions of retinoic acid is not reproduced in full by all bioactive synthetic retinoids.

10   Medical professionals have become very interested in the medicinal applications of retinoids. Among their uses approved by the FDA is the treatment of severe forms of acne and psoriasis. A large body of evidence also exists that these compounds can be used to arrest and, to an extent, reverse the

15   effects of skin damage arising from prolonged exposure to the sun. Other evidence exists that these compounds may be useful in the treatments of a variety of severe cancers including melanoma, cervical cancer, some forms of leukemia, and basal and squamous cell carcinomas. Retinoids have also shown an ability to be

20   efficacious in treating premalignant cell lesions, such as oral leukoplakia, and to prevent the occurrence of malignancy.

Use of the retinoids is associated with a number of significant side effects. The most serious of these is that, as a class, they are among the most potent teratogens known.

25   Teratogens are compounds that cause severe birth defects during specific periods of fetal exposure. Other side effects include irritation of the tissues treated, which can be so severe that patients cannot tolerate treatment.

Various investigations have been undertaken to

30   elucidate the structure-activity relationships governing the abilities of synthetic retinoids to induce the various

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pharmacological consequences of retinoic acid exposure. This has been a complicated task, however, since the assays available to investigators have been bioassays, carried out either in intact animals or in isolated tissues. Technical constraints have often

5   dictated the use of different small animal species for different assays. Interpretation of results has been complicated by possible pharmacokinetic and metabolic effects and possible species differences in the receptors involved. Nevertheless, definite differences in the pharmacological effects of various

10 synthetic retinoids have been observed.

Major insight into the molecular mechanism of retinoic acid signal transduction was gained in 1988. Prior to that time, several high abundance cellular retinoid binding proteins were incorrectly inferred to be the signal transducing receptors for

15 retinoic acid. In 1988, a member of the steroid/thyroid hormone intracellular receptor superfamily (Evans, *Science,* 240:889-95 (1988)) was shown to transduce a retinoic acid signal (Giguere et al., *Nature,* 330:624-29 (1987); Petkovich *et al., Nature,* 330: 444-50 (1987)). This unexpected finding related retinoic acid to other

20 non-peptide hormones and elucidated the mechanism of retinoic acid effects in altering cell function. It is now known that retinoids regulate the activity of two distinct intracellular receptor subfamilies; the Retinoic Acid Receptors (RARs) and the Retinoid X Receptors (RXRs).

25   The first retinoic acid receptor identified, designated RAR-alpha, acts to modulate transcription of specific target genes in a manner which is ligand-dependent, as has been shown to be the case for many of the members of the steroid/thyroid hormone intracellular receptor superfamily. The endogenous low-

30 molecular-weight ligand upon which the transcription-modulating

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activity of RAR-alpha depends is all-*trans*-retinoic acid.
Retinoic acid receptor-mediated changes in gene expression result
in characteristic alterations in cellular phenotype, with
consequences in many tissues manifesting the biological response

5   to retinoic acid. Two additional genes closely related to RAR-
alpha were recently identified and were designated RAR-beta and
RAR-gamma and are very highly related (Brand *et al., Nature*, 332:850-
53 (1988); Ishikawa *et al., Mol. Endocrin.*, 4:837-44 (1990)). In the
region of the retinoid receptors which can be shown to confer

10   ligand binding, the primary amino acid sequences diverge by less
than 15% among the three RAR subtypes or isoforms. All-*trans*-
retinoic acid is a natural ligand for the retinoic acid receptors
(RARs) and is capable of binding to these receptors with high
affinity, resulting in the regulation of gene expression. The

15   newly-discovered retinoid metabolite, 9-*cis*-retinoic acid, is also
. an activator of RARs.

      A related but unexpected observation was made recently
(Mangelsdorf *et al., Nature,* 345:224-29 (1990)), in which another
member of the steroid/thyroid receptor superfamily was also shown

20   to be responsive to retinoic acid. This new retinoid receptor
subtype has been designated Retinoid X Receptor (RXR), because
certain earlier data suggested that a derivative of all-*trans*-
retinoic acid may be the endogenous ligand for RXR. Like the
RARs, the RXRs are also known to have at least three subtypes or

25   isoforms, namely RXR-alpha, RXR-beta, and RXR-gamma, with
corresponding unique patterns of expression (Manglesdorf et al.,
Genes & Devel., 6:329-44 (1992)).

      Although both the RARs and RXRs respond to all-*trans*-
retinoic acid *in vivo*, the receptors differ in several important

30   aspects. First, the RARs and RXRs are significantly divergent in

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primary structure (*e.g.*, the ligand binding domains of RARα and RXRα have only 27% amino acid identity). These structural differences are reflected in the different relative degrees of responsiveness of RARs and RXRs to various vitamin A metabolites and synthetic retinoids. In addition, distinctly different patterns of tissue distribution are seen for RARs and RXRs. For example, in contrast to the RARs, which are not expressed at high levels in the visceral tissues, RXRα mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine. Finally, the RARs and RXRs have different target gene specificity. For example, response elements have recently been identified in the cellular retinal binding protein type II (CRBPII) and apolipoprotein AI genes which confer responsiveness to RXR, but not RAR. Furthermore, RAR has also been recently shown to repress RXR-mediated activation through the CRBPII RXR response element (Manglesdorf *et al.*, *Cell*, 66:555-61 (1991)). These data indicate that two retinoic acid responsive pathways are not simply redundant, but instead manifest a complex interplay. Recently, Heyman *et al.* (*Cell*, 68:397-406 (1992)) and Levin *et al.* (*Nature*, 355:359-61 (1992)) independently demonstrated that 9-*cis*-retinoic acid is a natural endogenous ligand for the RXRs. 9-*cis*-retinoic acid was shown to bind and transactivate the RXRs, as well as the RARs, and therefore appears to act as a "bifunctional" ligand.

In view of the related, but clearly distinct, nature of these receptors, ligands which are more selective for the Retinoid X Receptor subfamily would be of great value for selectively controlling processes mediated by one or more of the RXR isoforms, and would provide the capacity for independent control of the physiologic processes mediated by the RXRs.

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Ligands which preferentially affect one or more but not all of the receptor isoforms also offer the possibility of increased therapeutic efficacy when used for medicinal applications.

5   The entire disclosures of the publications and references referred to above and hereafter in this specification are incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention is directed to compounds, compositions, and methods for modulating processes mediated by

10   one or more Retinoid X Receptors. More particularly, the invention relates to compounds which selectively or preferentially activate Retinoid X Receptors, in comparison to Retinoic Acid Receptors. These compounds selectively modulate processes mediated by Retinoid X Receptors. Accordingly, the

15   invention also relates to methods for modulating processes selectively mediated by one or more Retinoid X Receptors, in comparison to Retinoic Acid Receptors, by use of the compounds of this invention. Examples of compounds used in and forming part of the invention include bicyclic benzyl, pyridinyl, thiophene,

20   furanyl, and pyrrole derivatives. Pharmaceutical compositions containing the compounds disclosed are also within the scope of this invention. Also included are methods for identifying or purifying Retinoid X Receptors by use of the compounds of this. invention.

25   ## BRIEF DESCRIPTION OF THE FIGURES

The present invention may be better understood and its advantages appreciated by those skilled in the art by referring to the accompanying drawings wherein:

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Figure 1 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCB.

Figure 2 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by all-*trans*-retinoic acid.

Figure 3 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 9-*cis*-retinoic acid.

Figure 4 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEB.

Figure 5 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-bromo-TTNEB.

Figure 6 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCHBP.

Figure 7 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEHBP.

Figure 8 presents the inhibition of transglutaminase activity by 9-*cis*-retinoic acid, all-*trans*-retinoic acid, and 3-methyl-TTNCB.

Figure 9 presents the Topical Dose Response, based on the test on Rhino mice, for 9-*cis*-retinoic acid, all-*trans*-retinoic acid, 3-methyl-TTNCB, 1, 25-dihydroxy Vitamin D.

Figure 10 presents the effect on rat HDL cholesterol of all-*trans*-retinoic acid, 9-*cis*-retinoic acid, 3-methyl-TTNCB, and 3-methyl-TTNEB.

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Figure 11 presents the concentration-related effect of
3-methyl-TTNEB and TTNPB individually on incorporation of
radiolabeled thymidine into DNA.

Figure 12 presents the concentration-related effect of
5   a combination of 3-methyl-TTNEB and TTNPB on incorporation of
radiolabeled thymidine into DNA.

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses retinoid-like compounds or ligands which have selective activity for members of the subfamily of Retinoid X Receptors (RXRs), in comparison to members of the subfamily of Retinoic Acid Receptors (RARs). Examples of such compounds are bicyclic benzyl, pyridinyl, thiophene, furanyl, and pyrrole derivatives which can be represented by the formulae:
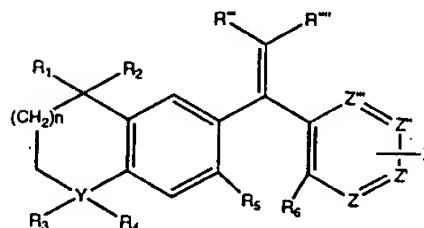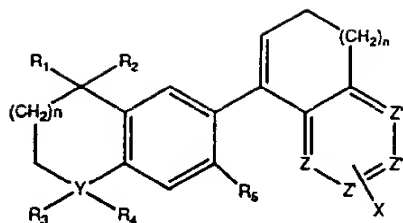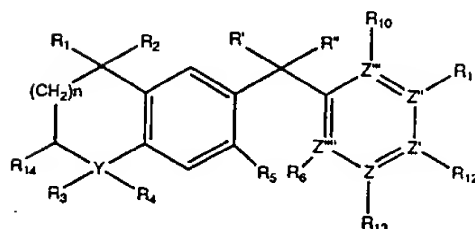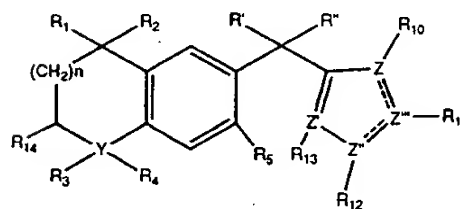

or

or

or

or

9

or

or
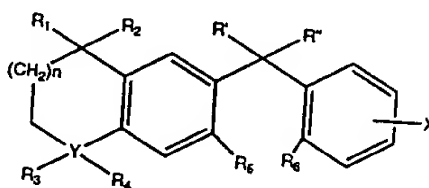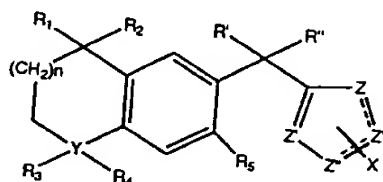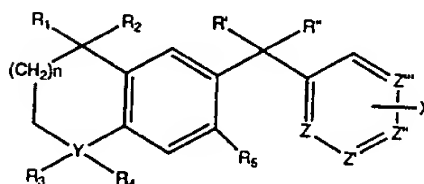
wherein

R_1 and R_2, each independently, represent hydrogen or lower
alkyl or acyl having 1-4 carbon atoms;

Y represents C, O, S, N, CHOH, CO, SO, SO_2, or a
pharmaceutically acceptable salt;

R_3 represents hydrogen or lower alkyl having 1-4 carbon atoms
where Y is C or N;

R_4 represents hydrogen or lower alkyl having 1-4 carbon atoms
where Y is C, but R_4 does not exist if Y is N, and neither R_3 or R_4
exist if Y is S, O, CHOH, CO, SO, or SO_2;

R' and R" represent hydrogen, lower alkyl or acyl having 1-4
carbon atoms, OH, alkoxy having 1-4 carbon atoms, thiol or thio
ether, or amino,

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or R′ or R″ taken together form an oxo (keto), methano, thioketo, HO-N=, NC-N=, (R_7R_8)N-N=, R_{17}O-N=, R_{17}N=, epoxy, cyclopropyl, or cycloalkyl group and wherein the epoxy, cyclopropyl, and cycloalkyl groups can be substituted with lower

5   alkyl having 1-4 carbons or halogen;

R′″ and R″″ represent hydrogen, halogen, lower alkyl or acyl having 1-4 carbon atoms, alkyl amino,

or R′″ and R″″ taken together form a cycloalkyl group having 3-10 carbons, and wherein the cycloalkyl group can be substituted

10   with lower alkyl having 1-4 carbons or halogen;

R_5 represents hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR_7, SR_7, NR_7R_8, or $(CF)_nCF_3$, but R_5 cannot be hydrogen if together R_6, R_{10}, R_{11}, R_{12} and R_{13} are all hydrogen, Z, Z′, Z″, Z″′, and Z″″ are all carbon, <u>and</u> R′ and R″ represent H, OH, C_1-

15   C_4 alkoxy or C_1-C_4 acyloxy or R′ and R″ taken together form an oxo, methano, or hydroxyimino group;

R_6, R_{10}, R_{11}, R_{12}, R_{13} each independently represent hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR_7, SR_7, NR_7R_8 or $(CF)_nCF_3$, and exist only if the Z, Z′, Z″, Z″′, or Z″″ from which it

20   originates is C, or each independently represent hydrogen or a lower alkyl having 1-4 carbons if the Z, Z′, Z″, Z″′, or Z″″ from which it originates is N, and where one of R_6, R_{10}, R_{11}, R_{12} or R_{13} is X;

R_7 represents hydrogen or a lower alkyl having 1-6 carbons;

25   R_8 represents hydrogen or a lower alkyl having 1-6 carbons;

R_9 represents a lower alkyl having 1-4 carbons, phenyl, aromatic alkyl, or q-hydroxyphenyl, q-bromophenyl, q-chlorophenyl, q-florophenyl, or q-iodophenyl, where q=2-4;

R_{14} represents hydrogen, a lower alkyl having 1-4 carbons, oxo,

30   hydroxy, acyl having 1-4 carbons, halogen, thiol, or thioketone;

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R_{17} represents hydrogen, lower alkyl having 1-8 carbons, alkenyl (including halogen, acyl, OR_7 and SR_7 substituted alkenes), R_9, alkyl carboxylic acid (including halogen, acyl, OR_7 and SR_7 substituted alkyls), alkenyl carboxylic acid (including halogen,

5 acyl, OR_7 and SR_7 substituted alkenes), alkyl amines (including halogen, acyl, OR_7 and SR_7 substituted alkyls), and alkenyl amines (including halogen, acryl, OR_7 and SR_7 substituted alkenes);

X is COOH, tetrazole, PO_3H, SO_3H, CHO, CH_2OH, $CONH_2$, COSH, $COOR_9$, $COSR_9$, $CONHR_9$, or COOW where W is a pharmaceutically

10 acceptable salt, and where X can originate from any C or N on the ring;

Z, Z', Z″, Z″′ and Z″″, each independently, represent C, S, O, N, or a pharmaceutically acceptable salt, but is not O or S if attached by a double bond to another such Z or if attached to

15 another such Z which is O or S, and is not N if attached by a single bond to another such Z which is N;

n = 0-3; and

the dashed lines in the second and seventh structures shown depict optional double bonds.

20 As used in this disclosure, pharmaceutically acceptable salts include but are not limited to: hydrochloric, hydrobromic, hydroiodic, hydrofluoric, sulfuric, citric, maleic, acetic, lactic, nicotinic, succinic, oxalic, phosphoric, malonic, salicylic, phenylacetic, stearic, pyridine, ammonium, piperazine,

25 diethylamine, nicotinamide, formic, urea, sodium, potassium, calcium, magnesium, zinc, lithium, cinnamic, methylamino, methanesulfonic, picric, tartaric, triethylamino, dimethylamino, and tris(hydroxymethyl)aminomethane. Additional pharmaceutically acceptable salts are known to those of skill in the art.

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Representative derivatives according to the present invention include the following:

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-methyl-TTNCB"**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-IPR-TTNCB"** or Compound **37**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated **"3-IPR-TTNEB"** or Compound **42**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated **"3-ethyl-TTNEB"** or Compound **45**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated **"3-bromo-TTNEB"** or Compound **46**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated **"3-chloro-TTNEB"** or Compound **43**;

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated **"3-methyl-TTNEB"**;

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p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-hydroxymethyl)]-benzoic acid, also known as 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)hydroxymethyl]benzoic acid, and designated **"3-methyl-TTNHMB"**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-bromo-TTNCB"** or Compound **41**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-chloro-TTNCB"** or Compound **38**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-hydroxy-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-hydroxy-TTNCB"** or Compound **39**;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated **"3-ethyl-TTNCB"** or Compound **40**;

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-thioketo)]-benzoic acid, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)thioketo]benzoic acid, and designated **"thioketone"**;

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-N-(4-hydroxyphenyl)benzamide, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]-N-(4-hydroxyphenyl)benzamide, and designated **"3-methyl-TTNCHBP"**;

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-methano)]-N-(4-hydroxyphenyl)benzamide, also known as 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]-N-(4-

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hydroxyphenyl)benzamide, and designated **"3-methyl-TTNEHBP"** or Compound **63**;

2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl]pyridine-5-carboxylic acid, designated **"TPNEP"** or Compound **58**;

ethyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-5-carboxylate, designated **"TPNEPE"** or Compound **Et-58**;

2-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl]pyridine-5-carboxylic acid, designated **"TTNEP"** or Compound **56**;

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) epoxy]benzoic acid, designated **"TPNEB"** or Compound **47**;

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) cyclopropyl]benzoic acid, designated **"TPNCB"** or Compound **48**;

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzenetetrazole, designated **"3-methyl-TTNEBT"** or Compound **55**;

5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-2-carboxylic acid, designated **"TPNEPC"** or Compound **60**;

2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]pyridine-5-carboxylic acid, designated **"TPNCP"** or Compound **62**;
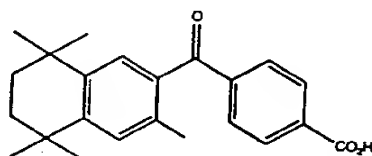
methyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]pyridine-5-carboxylate, designated Compound **Me-62**;

2-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl) cyclopropyl] pyridine-5-carboxylic acid, designated Compound **111**;
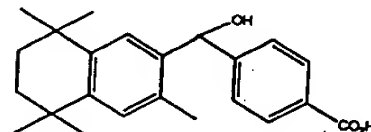
4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) carbonyl] benzoic acid oxime, designated Compound **112**; and

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4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) carbonyl] benzoic acid methyloxime, designated Compound **115**.
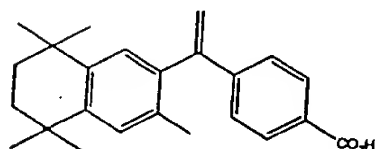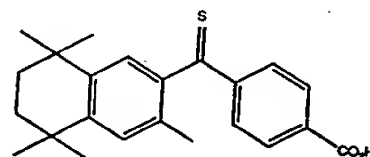
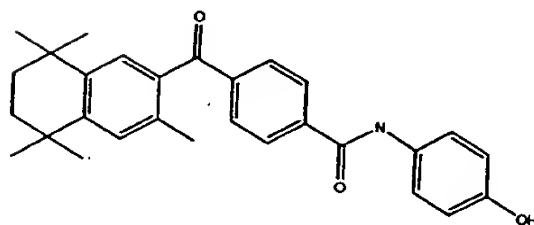Representative structures for such compounds are as follows:
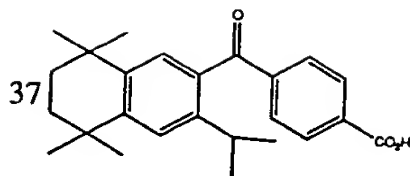
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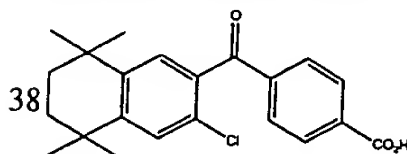
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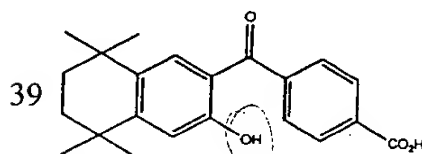
3-methyl-TTNEB

thioketone

3-methyl-TTNCHBP

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4-[(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzoic acid (3-iPr-TTNCB)

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4-[(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzoic acid (3-chloro-TTNCB)

39

4-[(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzoic acid (3-hydroxy-TTNCB)

40
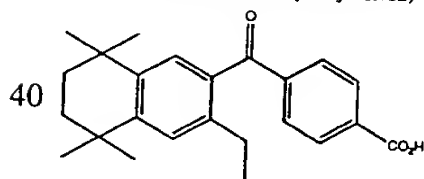
4-[(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzoic acid (3-Et-TTNCB)

41
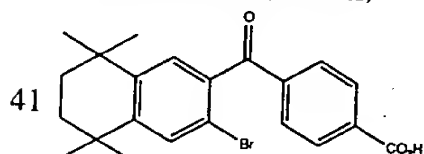
4-[(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzoic acid (3-bromo-TTNCB)

42
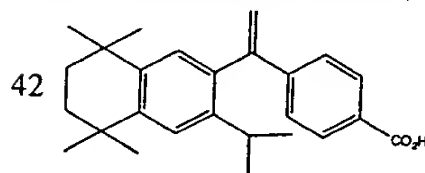
4-[1-(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] benzoic acid (3-iPr-TTNEB)

43

4-[1-(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] benzoic acid (3-chloro-TTNEB)

44

4-[1-(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl) ethenyl] benzoic acid (3-hydroxy-TTNEB)

45
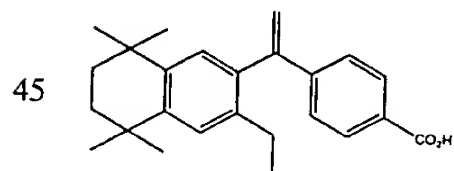
4-[1-(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] benzoic acid (3-Et-TTNEB)

46
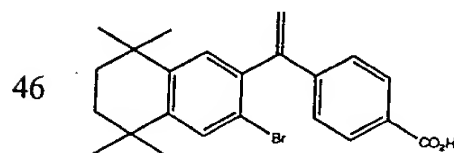
4-[1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] benzoic acid (3-bromo-TTNEB)

47
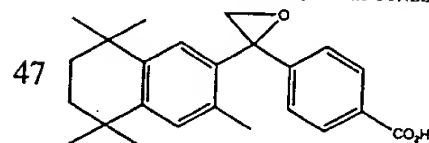
4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)epoxy] benzoic acid (TPNEB)

48
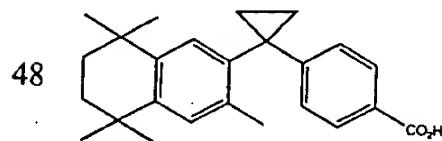
4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)cyclopropyl] benzoic acid (TPNCB)

17

49
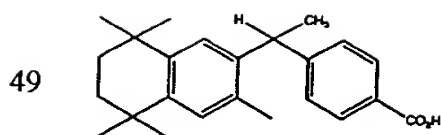
4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethyl] benzoic acid (PTNEB)

50

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
methylidine cyclopentane] benzoic acid (PTNCB)

51

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)-2-methyl propenyl] benzoic acid (PTNIB)

52

2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] thiophene-5-carboxylic acid (TTNCTC)

53
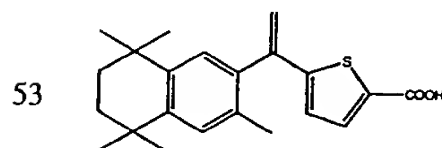
2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] thiophene-5-carboxylic acid (TTNETC)

54

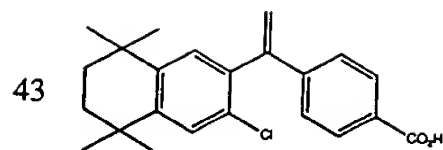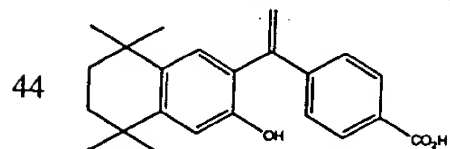4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] benzenetetrazole (3-methyl-TTNCBT)

55

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] benzenetetrazole (3-methyl-TTNEBT)

56

2-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] pyridine-5-carboxylic acid (TTNEP)

57

2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] pyridine-5-carboxylic acid

58

2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] pyridine-5-carboxylic acid (TPNEP)

Et-58

Ethyl-2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)ethenyl] pyridine-5-carboxylate

59

5-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)carbonyl] pyridine-2-carboxylic acid (TTNCP)

18

60

5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] pyridine-2-carboxylic acid (TPNEPC)

63

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(4-hydroxyphenyl)benzamide (3-Me-TTNEHBP)

61

Ethyl-5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)ethenyl] pyridine-2-carboxylate (3TTNEPE)

64

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(4-fluorophenyl)benzamide (3-Me-TTNEFBP)

62

2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)cyclopropyl] pyridine-5-carboxylic acid (TPNCP)

65

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(4-carboxyphenyl)benzamide (3-Me-TTNECBP)

Me-62

Methyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl)cyclopropyl] pyridine-5-carboxylate

66

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl]-N-(3-hydroxyphenyl)benzamide (3-Me-m-TTNCHBP)

Et-62

Ethyl-2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)cyclopropyl] pyridine-5-carboxylate

67

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(3-hydroxyphenyl)benzamide (3-Me-m-TTNEHBP)

19

68

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(2-hydroxyphenyl)benzamide(3-Me-o-TTNCHBP)

71

4-[1-(3,5,5,6,7,7-hexamethyl-2-indanyl)ethenyl] benzoic acid

69

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
ethenyl]-N-(3-carboxyphenyl)benzamide (3-Me-m-TTNECBP) CO₂H

72

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
methyl] benzoic acid

70

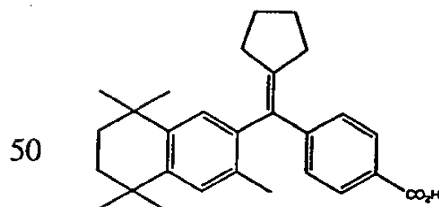4-[1-(3,5,5,7,7-pentamethyl-2-indanyl)ethenyl] benzoic acid

Et-3-methyl-
TTNEB

Ethyl-4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)ethenyl] benzoate

20

111

2-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl)cyclopropyl] pyridine-5-carboxylic acid

114

2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
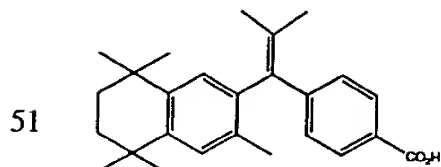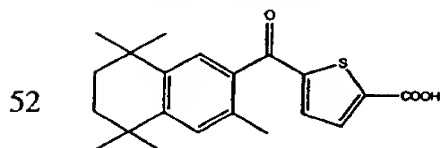2-naphthyl)carbonyl] pyridine-5-carboxylic acid oxime

112

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-
2-naphthyl) carbonyl] benzoic acid oxime

115

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid methyloxime

Et-112

Ethyl-4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)carbonyl] benzoate oxime

Et-115

Ethyl-4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)carbonyl] benzoate methyloxime

113

4-[(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthyl) carbonyl] benzoic acid oxime

116

2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] pyridine-5-carboxylic acid methyloxime

21

138

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
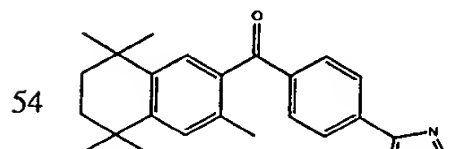carbonyl] benzoic acid butyloxime

141

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid allyloxime

139

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid propyloxime

142

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid 4-(3-methyl but-2-enoic acid)oxime

140

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid cyanoimine

143

4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)
carbonyl] benzoic acid 1-amino ethyloxime

22

In addition, thiophene, furanyl, pyridine, pyrazine, pyrazole, pyridazine, thadiazole, and pyrrole groups function as isosteres for phenyl groups, and may be substituted for the phenyl group of the above bicyclic benzyl derivatives.

5   Representative derivatives of the present invention can be prepared according to the following illustrative synthetic schemes:
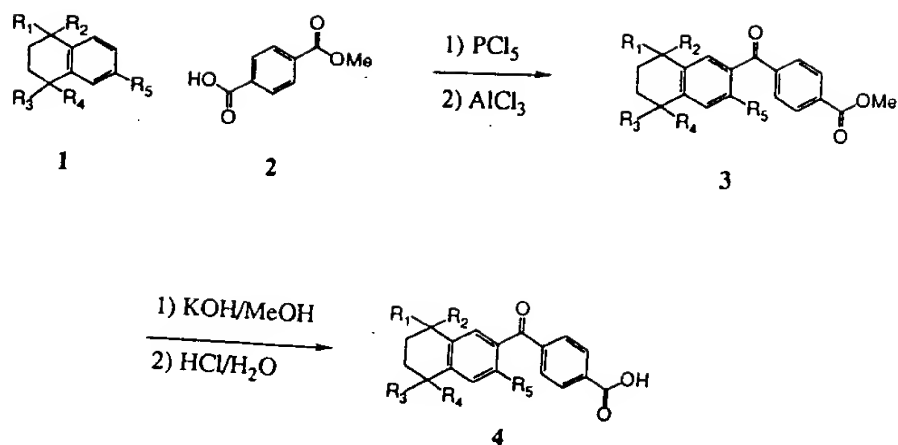
Compounds of structure **1** containing R_5 = lower alkyl are prepared in accordance with United States Patent No. 2,897,237. When R_5 = Halo, OH, amino, or thio, the products are prepared by

5   standard Friedel-Crafts reaction conditions combining the appropriate substituted benzene with 2,5-dichloro-2,5-dimethyl hexane in the presence of aluminum trichloride.

Condensation of **1** with mono-methyl terephthalate **2** was carried out by addition of PCl_5 to **1** and **2** in CH_2Cl_2 followed by addition of

10     $AlCl_3$ at room temperature.

The resulting methyl esters **3** are hydrolyzed to the carboxylic acid **4** by refluxing in aqueous KOH-MeOH followed by acidification.

24

4     $\xrightarrow[\text{2) HCl/H}_2\text{O}]{\text{1) NaBH}_4\text{/MeOH}}$

5

3     $\xrightarrow[\text{NaNH}_2]{(C_6H_5)_3P^+\text{-}CH_3Br^-}$

6

$\xrightarrow[\text{2) HCl/H}_2\text{O}]{\text{1) KOH/MeOH}}$

7
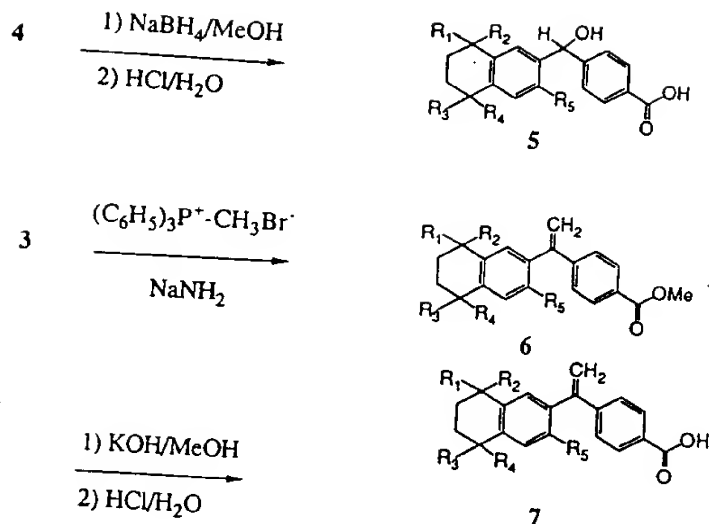
Treatment of ketone **4** with NaBH$_4$ afforded alcohol **5**.

Treatment of the methyl ester **3** with methyltriphosphonium bromide-sodium amide in THF afforded the methano compound **6**.

The carboxylic acid **7** was formed by adding KOH to methano compound **6** in MeOH, followed by acidification.

25
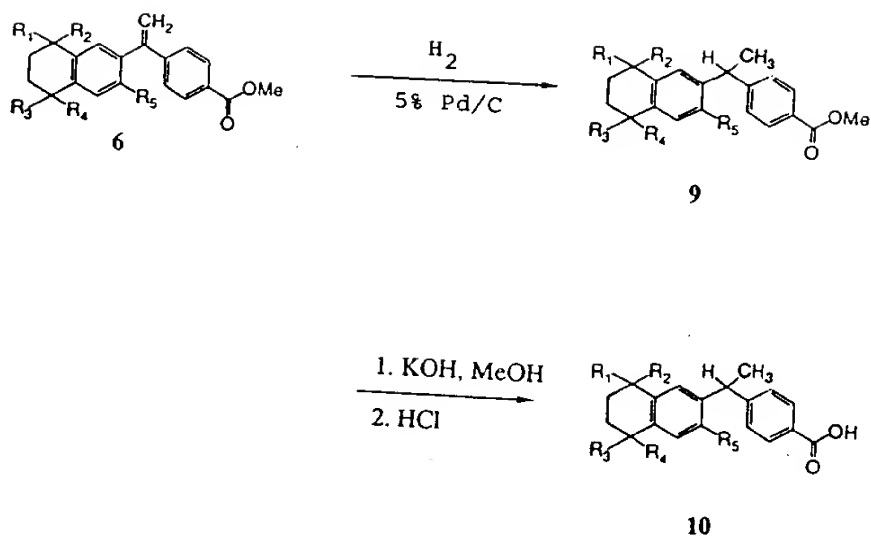
Treatment of the methyl ester **6** with hydrogen gas and 5%
palladium over carbon in ethyl acetate yields the hydrogenated
compound **9**.

Treatment of compound **9** with aqueous KOH in refluxing
MeOH, followed by acidification, yields the carboxylic acid
compound **10**.

11

12
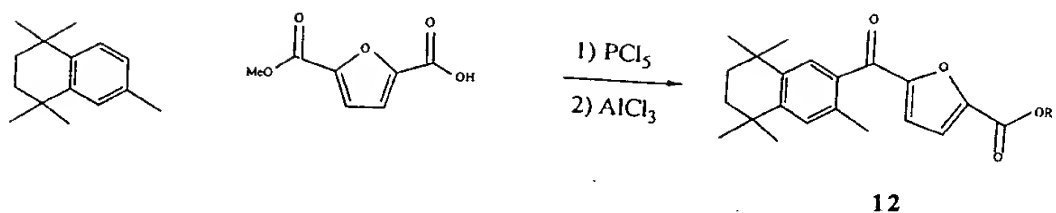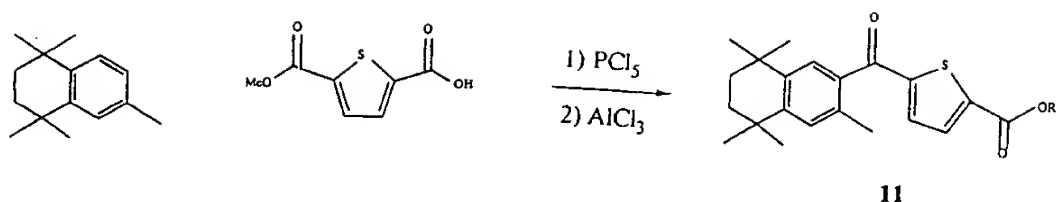
R = Me or H

Condensation of 1 with thiophene 2,5-mono methyl dicarboxylic acid or furanyl 2,5-mono methyl dicarboxylic acid was carried out by addition of PCl_5 in CH_2Cl_2 followed by addition of AlCl₃ at room temperature to give esters **11** and **12**, which were hydrolyzed with KOH followed by acidification to the corresponding acids.

5

27

13

14
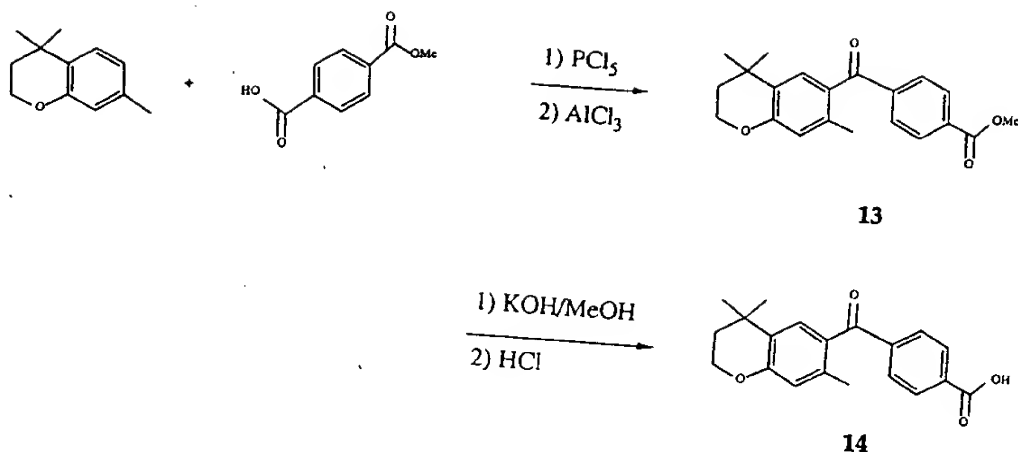
4,4-Dimethylchroman and 4,4-dimethyl-7-alkylchroman compounds of type **13** and **14** as well as 4,4-dimethylthiochroman,

5   4,4-dimethyl-7-alkylthiochroman, 4,4-dimethyl-1,2,3,4-tetrahydroquinoline, and 4,4-dimethyl-7-alkyl-1,2,3,4-tetrahydroquinoline analogs were synthesized by similar methods as compound **3**, *i.e.*, Friedel-Crafts conditions combining the appropriate dimethylchroman, dimethylthiochroman or

10   dimethyltetrahydroquinoline with mono-methyl terephthalate acid chloride in the presence of $AlCl_3$ or $SnCl_4$, followed by base hydrolysis and acidification to give the carboxylic acid. For the synthesis of the tetrahydroquinoline analogs, it was necessary to acylate the amine before Friedel-Crafts coupling with mono-methyl

15   terephthalate acid chloride. For the synthesis of the appropriate dimethylchromans, dimethylthiochromans and tetrahydroquinolines, see U.S. Patent Nos. 5,053,523 and 5,023,341 and European Patent Publication No. 0284288.

28
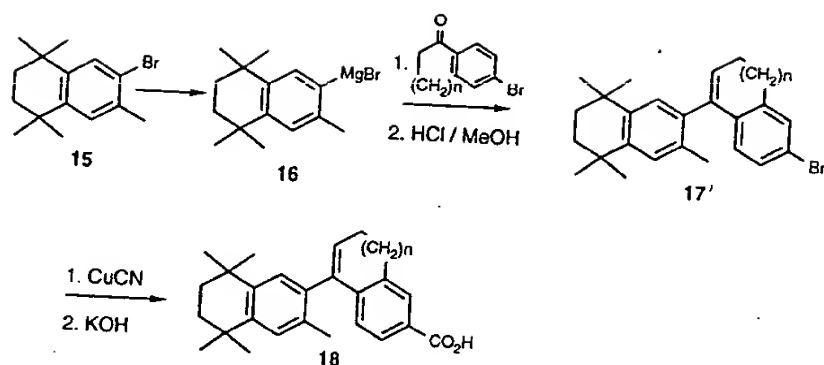
Compounds of the type **18** were synthesized by nucleophillic addition
of the Grignard reagent **16** to bromotetralone, bromoindane, or other

5 bicyclic ketone derivitive. Treatment of the resulting alcohol
with methanolic HCl gave the intermediate **17**. Displacement of the
bromine with CuCN in quinoline gave the nitrile which was then
hydrolyzed to the acid **18** in refluxing KOH. Bromine compound **15**
was synthesized from 2,5-dichloro-2,5-dimethylhexane and 2-

10 bromotoluene with a catalytic amount of AlCl$_3$.
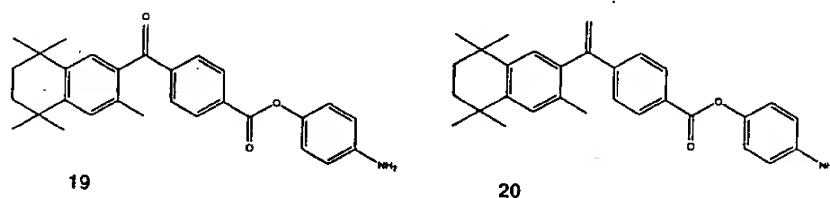
Treatment of compounds 3-methyl-TTNCB and 3-methyl-TTNEB with DCC,
p-aminophenol, and DMAP resulted in the amino-esters **19** and **20**.
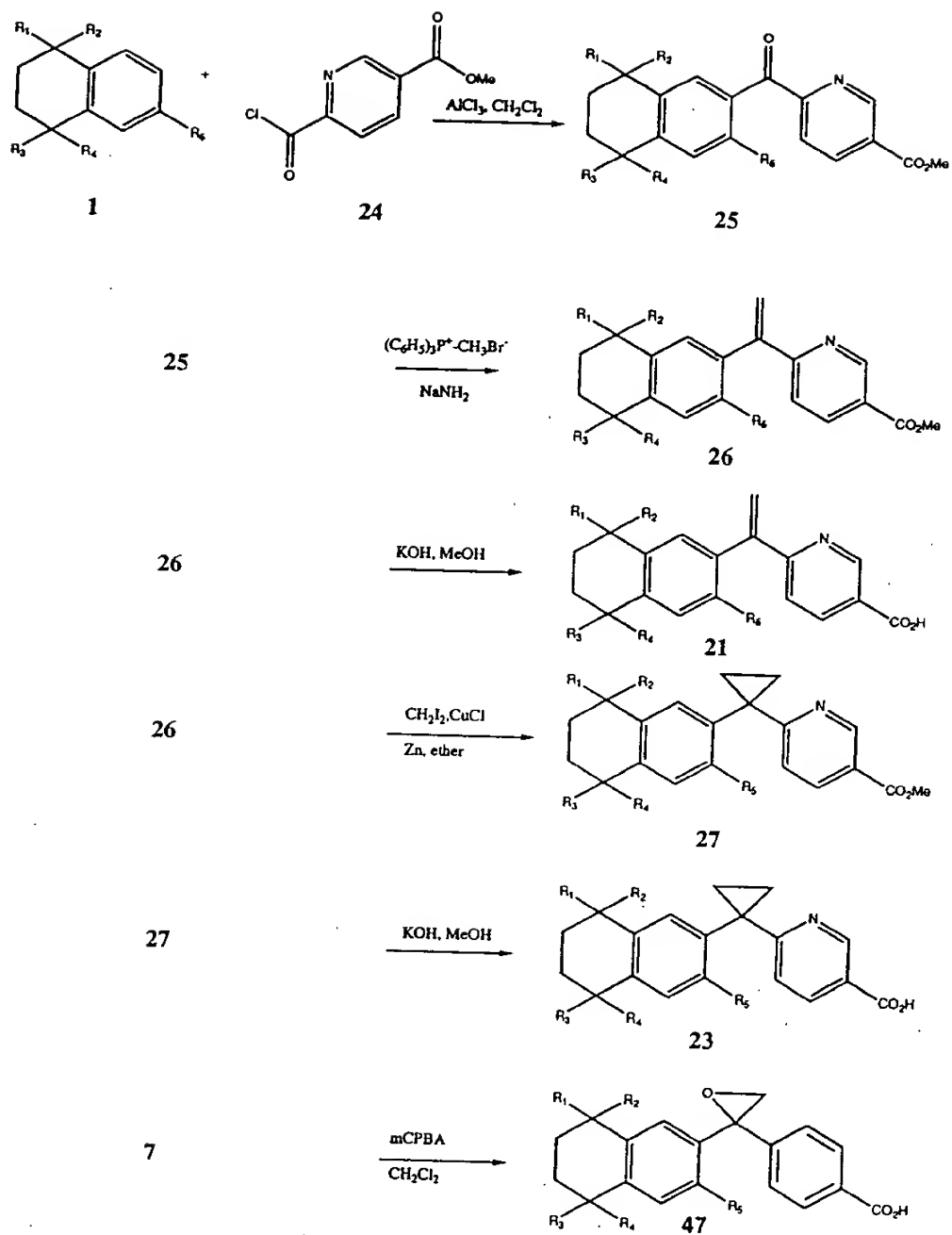
Representative pyridinal derivatives (compounds **21, 23,**
26, and **27)** may be prepared according to the illustrative synthetic
schemes shown above. The synthesis of compound **21** is similar to
that previously described for compound **7.** Pentamethyl
5   tetrahydronaphthalene **1,** pyridinal acid chloride **24,** and AlCl$_3$ are
stirred in CH$_2$Cl$_2$ to give the ketone **25.** Treatment of the ketone **25**
with methyl triphosphonium bromide-sodium amide in THF afforded the
ethenyl compound **26.** Hydrolysis of **26** (KOH,MeOH) followed by
acidification gave the acid **21.** The cyclopropyl analog **23** was
10   synthesized by treatment of the ethenyl compound **26** with CH$_2$I$_2$, zinc
dust, CuCl in refluxing ether (Simmons-Smith reaction). Hydrolysis
of the resulting cyclopropyl ester **27** was achieved with methanolic
KOH followed by acidification to give compound **23.** When R$_1$-R$_5$ are
methyl, for example, compound **62 (TPNCP)** is obtained, as shown in
15   Example 33 below.

Other cyclopropyl derivatives such as **TPNCB** (compound **48)**
may be likewise prepared by the same method as described for analog
23: olefin **6** is treated with the Simmons-Smith reagent described
above, followed by hydrolysis with methanolic-KOH and acidification
20   **(HCI)** to give the desired cyclopropyl derivative. Epoxy
derivatives such as **TPNEB** (compound **47)** may be synthesized by
treatment of compound **7** with m-chloroperbenzoic acid at room
temperature in CH$_2$Cl$_2$ for several hours.

Alternatively, pyridinal analogs, such as compounds **58**
25   **(TPNEP), 60 (TPNEPC),** and **61 (3TTNEPE),** may be prepared by the
following synthetic route.

31

Scheme (compounds 29–32):

29 — CO₂H / CO₂H pyridine diacid
SOCl₂ / MeOH / > 95%
30 — CO₂Me / CO₂Me
KOH / MeOH / > 95%
31 — CO₂K / CO₂Me
1. SOCl₂ 2. Me₂CuLi / > 56%
32 — acetyl pyridine / CO₂Me

Scheme (compounds 33, 34, 58, Et-58):

Cl / Cl + Br-toluene
AlCl₃ / > 95%
33 — tetramethyltetrahydronaphthalene Br
1. n-BuLi 2. 32 / > 60%
34 — OH / CO₂Me
1. H⁺ 2. KOH / > 90%
58 — CO₂H
H⁺-EtOH > 95%
Et-58 — CO₂Et

Scheme (compounds 31, 36, 36A):

31 — CO₂K / CO₂Me
1. SOCl₂ 2. NHiPr₂ / > 90%
36 — CONiPr₂ / CO₂Me
1. KOH 2. SOCl₂ 3. Me₂CuLi / > 60%
36A — CONiPr₂ / acetyl

Scheme (compounds 33, 34, 35, 60, 61):

33 — Br tetramethyltetrahydronaphthalene
1. n-BuLi 2. 36A
H⁺
34 — CONiPr₂ / > 80%
DIBAL / 83%
35 — CHO
H₂O₂ / > 90%
60 — CO₂H
H⁺, EtOH / > 95%
61 — CO₂Et

32

Synthesis of Oximes and Methyloximes

Oxime from Carboxylic Acid:

py / EtOH

NH$_2$OH-HCl
90 - 95%

3-methyl TTNCB

112

Oximes from Esters:

NH$_2$OH-HCl

KOH
MeOH
90-95%

41 X = CH, R = Br
57 X = N, R = CH$_3$, R$_1$ = CH$_3$
R$_1$ = Me, Et, iPr

113 X = CH, R = Br
114 X = N, R = CH$_3$

Methyloximes:

py / EtOH

NH$_2$OCH$_3$-HCl
90%

3-methyl TTNCB X = CH
57 X = N

115 X = CH
116 X = N

33
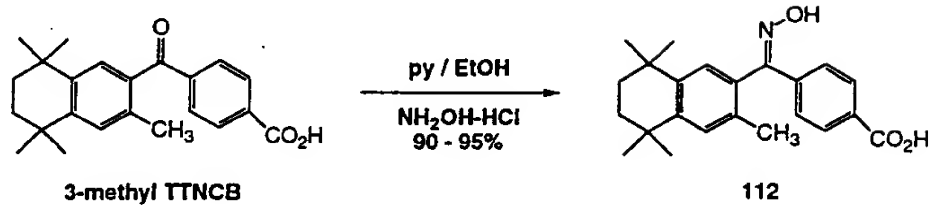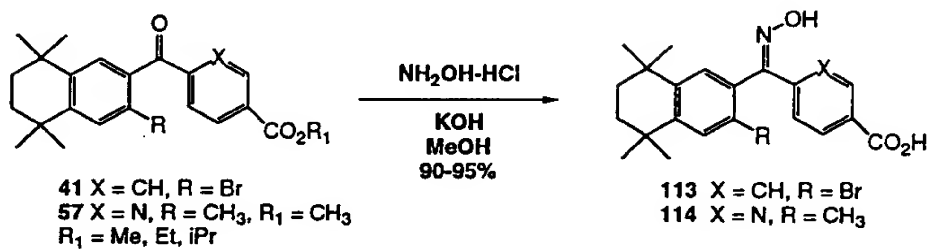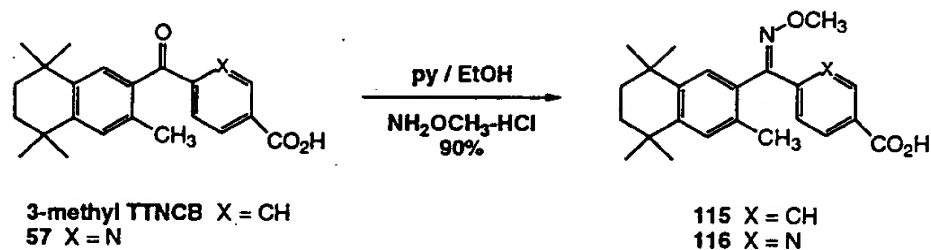
Synthesis of Alkyloximes

112

138 R = butyl
139 R = propyl
141 R = allyl

142 R = (structure shown with H₃C, CO₂H)

NaH
DMPU / THF

R-Br

112

BrCH₂CH₂NH₂ · HBr
DMF / excess KOH

pH = 2
50%

143
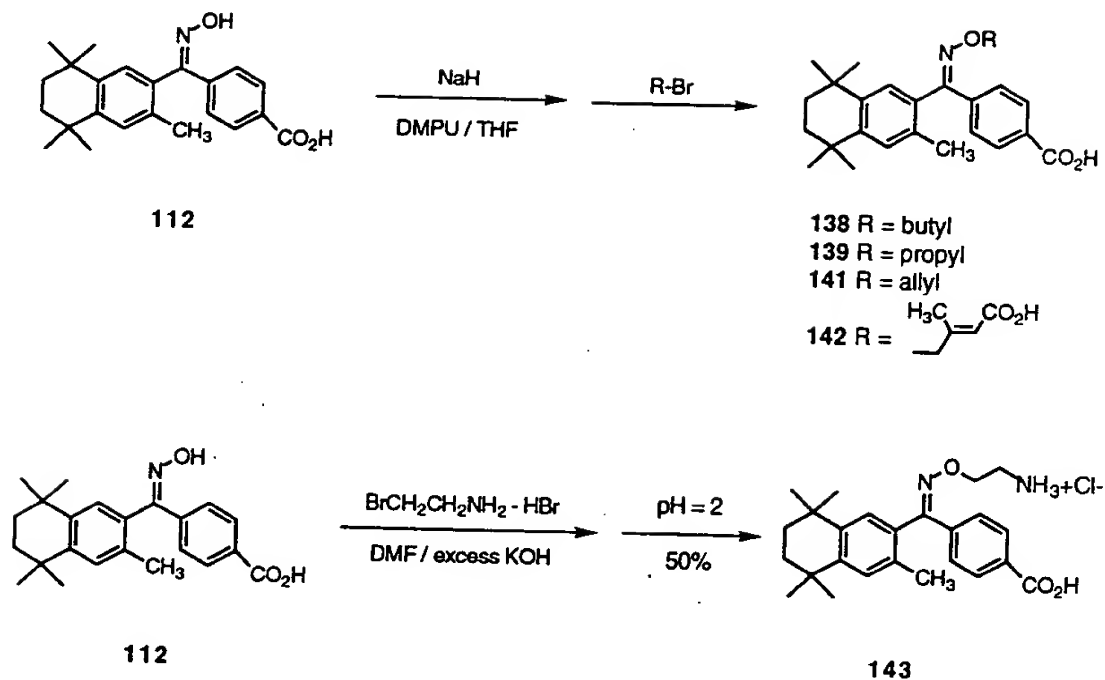
Synthesis of Cyanoimine

3-methyl TTNCB

TMS-N=C=N-TMS
CH₂Cl₂   TiCl₄

140

34
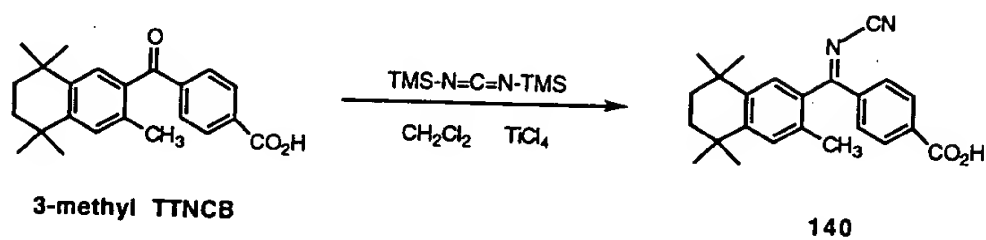
Representative oxime derivatives (compounds **112, 113,** and
114) may be prepared according to the illustrative synthetic
schemes shown above. Synthesis of a representative methyloxime
(compound **115**) is also shown. A ketone such as 3-methyl-TTNCB is
5   treated with hydroxylamine hydrochloride in pyridine and heated at
reflux to give oxime **112**. Alkyl oxime ethers are prepared from the
corresponding ketone (such as 3-methyl-TTNCB) by treatment with
methoxylamine hydrochloride in refluxing pyridine to give the
methoxyomine **116**. Also see Examples 44-49, below. **Et-115** (and
10   other ethyl esters such as **Et-58, Et-62, Et-3-methyl-TTNEB**) can be
made by treatment of the respective carboxylic acids with oxalyl
chloride to form the acid chloride, followed by treatment with EtOH
and pyridine to give the ethyl ester. The oxime **Et-112** can be made
from Et-4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
15   naphthyl)carbonyl] benzoate (**Et-3-methyl TTNCB**) by treatment with
pyridine/EtOH and $NH_2OH-CHl$ at reflux.

Substituted oximes (compounds **138-143**) may also be
prepared as shown above. These compounds were synthesized from the
corresponding free oxime (**112**) by treatment of the oxime with NaH
20   followed by alkylation with the appropriate bromo alkyl group (R-
Br).

35

Illustrative examples for the preparation of some of the compounds according to this invention are as follows:

Example 1

Preparation of compound **3** where R_1, R_2, R_3, R_4 and R_5 are methyl, R'
5 and R" are oxo, and X=COOMe:

To 7 gm (34.7 mmol) of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene and 6 gm (33.3 mmol) of mono-methyl teraphthalate in 200 mL of CH_2Cl_2 was added 8 g (38.8 mmol) of PCl_5. The reaction boiled vigorously and turned clear within 10 min.
10 After stirring for an additional 1 h, 6 g (43.5 mmol) of $AlCl_3$ was added in 1 g portions over 15 min. and the reaction was allowed to stir overnight. The mixture was poured into 300 mL of 20% aqueous HCl and extracted with 5% EtOAc-hexanes, dried (MgSO4), concentrated, and crystallized from MeOH to give ca. 6 gm (16.5
15 mmol) of methyl ester **3**.

^{1}HNMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 3.93 (s, $COOCH_3$), 7.21 (s, Ar-CH), 7.23 (s, Ar-CH), 7.85 (d, J=8 Hz, Ar-2(CH)), 8.18 (d, J=8 Hz, Ar-2(CH)).

Example 2

20 Preparation of compound **4** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are oxo, and X = COOH (**3-methyl-TTNCB**):

To 6 gm (16.5 mmol) of methyl ester **3** suspended in 100 mL of MeOH was added 50 mL of 5N aqueous KOH. The mixture was heated under reflux for 1 h, cooled, acidified (20% aqueous HCl) and the
25 organics extracted with EtOAc. After drying (MgSO4), the product was concentrated and precipitated from 1:4 EtOAc-hexanes to give ca. 5 g (14.3 mmol) of acid **4**.

^{1}HNMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 7.21 (s, Ar-CH), 7.23 (s, Ar-CH), 7.91 (d,
30 J=8 Hz, Ar-2(CH)), 8.21 (d, J=8 Hz, Ar-2(CH)).

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Example 3

Preparation of compound **5** where R_1, R_2, R_3, R_4 and R_5 are methyl, R′ = H and R" = OH, and X = COOH **(3-methyl-TTNHMB)**:

To a 1:1 THF-MeOH solution containing 1 g (2.86 mmol) of
5   ketone **4** was added 100 mg of $NaBH_4$. The mixture was heated to 50°C for 10 min., cooled, acidified (20% aqueous HCl), and the organics extracted (EtOAc). After drying ($MgSO_4$), the product was concentrated and precipitated from 1:3 EtOAc-hexanes to give 550 mg (1.56 mmol) of the alcohol **5**.

10   ^{1}HNMR (CD_3OCD_3) δ 1.20 (s, CH_3)), 1.22 (s,(CH_3)), 1.22 (s, 2(CH_3)), 1.65 (s, 2(CH_2)), 2.21 (s, CH_3), 6.00 (s, -CHOH-), 7.09 (s, Ar-CH), 7.41 (s, Ar-CH), 7.53 (d, J=8 Hz, Ar-2(CH)), 8.01 (d, J=8 Hz, Ar-2(CH)).

Example 4

15   Preparation of compound **6** where R_1, R_2, R_3, R_4 and R_5 are methyl, R′ and R" are methano, and X = COOMe:

To 1 gm of methyl ester **3** (2.7 mmol) in 25 mL of dry THF was added 1.2 g (3.08 mmol) of methyltriphosphonium bromide-sodium amide. The solution was stirred at RT for 3 h or until complete by
20   TLC (20% EtOAc-hexanes). Water was added and the organics were extracted with EtOAc, dried ($MgSO_4$), concentrated and purified by SiO_2 chromatography (5% EtOAc-hexanes) followed by crystallization from MeOH to give 700 mg (1.93 mmol) of methano compound **6**.

^{1}HNMR (CD_3OCD_3) δ 1.22 (s, 2(CH_3)), 1.30 (s, 2(CH_3)), 1.72 (s,
25   2(CH_2)), 1.95 (s, CH_3), 3.85 (s, $COOCH_3$), 5.29 (s,=CH), 5.92 (s, =CH), 7.19 (s, Ar-CH), 7.20 (s, Ar-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J=8 Hz, Ar-2(CH)).

Example 5

Preparation of compound **7** where R_1, R_2, R_3, R_4 and R_5 are methyl, R'
and R" are methano, and X = COOH **(3-methyl-TTNEB)**:

5   To 500 mg of methano compound **6** (1.38 mmol) in 20 mL of
MeOH was added 5 mL of 5 N aqueous KOH and the suspension was
refluxed for 1 h. After acidification (20% aqueous HCl) the
organics were extracted (EtOAc), dried ($MgSO_4$), concentrated, and
the solids recrystallized from EtOAc-hexanes 1:5 to give 350 mg
(1.0 mmol) of the carboxylic acid **7**.

10 ^{1}HNMR (CD_3OCD_3), δ 1.22 (s, 2(CH_3)), 1.30 (s, 2(CH_3)), 1.72 (s,
2(CH_2)), 1.95 (s, CH_3), 5.22 (s,=CH), 5.89 (s,=CH), 7.19 (s, Ar-CH),
7.20 (s, Ar-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J = 8 Hz, Ar-
2(CH)).

Example 6

15 Preparation of compound **37** where R_1,R_2,R_3,R_4 are methyl, R_5 is
isopropyl, R' and R" are oxo, and X=COOH **(3-IPR-TTNCB)**:

The compound was prepared in a manner similar to that of
compound **4** except that 6-isopropyl-1,1,4,4-tetramethyl-1,2,3,4-
tetra-hydronaphthalene was substituted for 1,1,4,4,6-pentamethyl-
20 1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 254 ˚C; ^{1}H-
NMR ($CDCl_3$) δ1.19 (d,J=7 Hz,CH(CH_3)$_2$), 1.21 (s,2(CH_3)), 1.33
(s,2(CH_3)), 1.70 (s,2(CH_2)), 3.12 (q,J=7 Hz,CH(CH_3)$_2$), 7.14 (s,Ar-
CH), 7.37 (s,Ar-CH), 7.92 (d,J=8 Hz, Ar-2(CH)), 8.18 (d,J=8 Hz, Ar-
2(CH)).

Example 7

25 Preparation of compound **38** where R_1,R_2,R_3,R_4 are methyl, R_5 is
chloro, R' and R" are oxo, and X=COOH **(3-chloro-TTNCB)**:

The compound was prepared in a manner similar to that of compound **4** except that 6-chloro-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 254 °C; ^{1}H-

5   NMR (CDCl$_3$) δ1.26 (s,2(CH$_3$)), 1.32 (s,2(CH$_3$)), 1.72 (s,2(CH$_2$)), 7.35 (s,Ar-CH), 7.36 (s,Ar-CH), 7.91 (d,J=8 Hz, Ar-2(CH)), 8.19 (d,J=8 Hz, Ar-2(CH)).

Example 8

Preparation of compound **39** where R$_1$,R$_2$,R$_3$,R$_4$ are methyl, R$_5$ is

10   hydroxy, R' and R" are oxo, and X = COOH **(3-hydroxy-TTNCB)**:

The compound was prepared in a manner similar to that of compound **4** except that 6-hydroxy-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 264 °C; ^{1}H-

15   NMR (CDCl$_3$) δ1.17 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.68 (s,2(CH$_2$)), 7.02 (s,Ar-CH), 7.44 (s,Ar-CH), 7.77 (d,J=8 Hz, Ar-2(CH)), 8.27 (d,J=8 Hz, Ar-2(CH)), 11.50 (s,-OH).

Example 9

Preparation of compound **40** where R$_1$,R$_2$,R$_3$,R$_4$ are methyl, R$_5$ is ethyl,

20   R' and R" are oxo, and X=COOH **(3-Et-TTNCB)**:

The compound was prepared in a manner similar to that of compound **4** except that 6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 226°C; ^{1}H-

25   NMR (CDCl$_3$) δ1.16 (t,J=7.5 Hz,-CH$_2$CH$_3$), 1.19 (s,2(CH$_3$)), 1.32 (s,2(CH$_3$)), 1.69 (s,2(CH$_2$)), 2.69(q,J=7.5 Hz, CH$_2$CH$_3$), 7.20 (s,Ar-CH), 7.25 (s,Ar-CH), 7.87 (brd,Ar-2(CH)), 8.20 (brd,Ar-2(CH)).

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Example 10

Preparation of compound **41** where R_1, R_2, R_3, R_4 are methyl, R_5 is bromo, R' and R" are oxo, and X = COOH **(3-bromo-TTNCB)**:

The compound was prepared in a manner similar to that of
5   compound **4** except that 6-bromo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 275 °C; ^{1}H-NMR (CDCl$_3$) δ1.25 (s,2(CH$_3$), 1.32 (s,2(CH$_3$)), 1.71 (s,2(CH$_2$)), 7.30 (s,Ar-CH), 7.54 (s,Ar-CH), 7.90 (d,J=8 Hz, Ar-2(CH)), 8.18 (d,J=8
10   Hz,Ar-2(CH)).

Example 11

Preparation of compound **42** where R_1, R_2, R_3, R_4 are methyl, R_5 is isopropyl, R' and R" are methano, and X = COOH **(3-IPR-TTNEB)**:

The compound was prepared in a manner similar to that of
15   compound **7** except that 6-isopropyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 252 °C; ^{1}H-NMR (CDCl$_3$) δ1.05 (d,J=7 Hz,CH(CH$_3$)$_2$), 1.27 (s,2(CH$_3$)), 1.32 (s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 2.73(q,J=7 Hz,CH(CH$_3$)$_2$), 5.32 (s,=CH),
20   5.87 (s,=CH) 7.06 (s,Ar-CH), 7.23 (s,Ar-CH), 7.40 (d,J=8 Hz,Ar-2(CH)), 8.040 (d,J=8 Hz,Ar-2(CH)).

Example 12

Preparation of compound **43** where R_1, R_2, R_3, R_4 are methyl, R_5 is chloro, R' and R" are methano, and X=COOH **(3-chloro-TTNEB)**:

25   The compound was prepared in a manner similar to that of compound **7** except that 6-chloro-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 233 °C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.71

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(s,2(CH$_2$)), 5.42 (s,=CH), 5.89 (s,=CH), 7.23 (s,Ar-CH), 7.28 (s,Ar-CH), 7.37 (d,J=8 Hz,Ar-2(CH)), 8.03 (d,J=8 Hz,Ar-2(CH)).

Example 13

Preparation of compound **44** where R$_1$,R$_2$,R$_3$,R$_4$ are methyl, R$_5$ is
hydroxy, R' and R" are methano, and X = COOH (**3-hydroxy-TTNEB**):

The compound was prepared in a manner similar to that of compound **7** except that 6-hydroxy-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 216 °C; ^{1}H-NMR (CDCl$_3$) δ1.21 (s,2(CH$_3$), 1.30 (s,2(CH$_3$)), 1.68 (s,2(CH$_2$)), 5.54 (s,=CH), 5.94 (s,=CH), 6.86 (s,Ar-CH), 7.00 (s,Ar-CH), 7.48 (d,J=8.4 Hz, Ar-2(CH)), 8.07 (d,J=8.4 Hz, Ar-2(CH)).

Example 14

Preparation of compound **45** where R$_1$,R$_2$,R$_3$,R$_4$ are methyl, R$_5$ is ethyl,
R' and R" are methano, and X = COOH (**3-Et-TTNEB**):

The compound was prepared in a manner similar to that of compound **7** except that 6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 236 °C; ^{1}H-NMR (CDCl$_3$) δ0.99 (t,J=7.6 Hz,-CH$_2$CH$_3$), 1.27 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 2.29(q,J=7.6 Hz,-CH$_2$CH$_3$), 5.34 (s,=CH), 5.83 (s,=CH), 7.08 (s,Ar-CH), 7.12 (s,Ar-CH), 7.38 (d,J=8 Hz, Ar-2(CH)), 8.00 (d,J=8 Hz, Ar-2(CH)).

Example 15

Preparation of compound **46** where R_1, R_2, R_3, R_4 are methyl, R_5 is bromo, R' and R" are methano, and X = COOH **(3-bromo-TTNEB)**:

The compound was prepared in a manner similar to that of

5   compound **7** except that 6-bromo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 235 °C; ^{1}H-NMR (CDCl$_3$) δ1.27 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.71 (s,CH$_3$), 5.40 (s,=CH), 5.90 (s,=CH), 7.26 (s,Ar-CH), 7.36 (s,Ar-CH), 7.43

10   (d,J=8 Hz,Ar-2(CH)), 8.04 (d,J=8 Hz,Ar-2(CH)).

Example 16

Preparation of compound **47** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R" taken together are CH$_2$-O (epoxide), and X = COOH **(TPNEB)**:

The compound was prepared from compound **6** where R_1, R_2,

15   R_3, R_4, R_5 are methyl. To 1 g (2.76 mmol) of olefin **6** in 5 mL of CH$_2$Cl$_2$ was added 600 mg (3.46 mmol) of mCPBA and the reaction was stirred at room temperature for 2 h. Water was added followed by extraction of the organics with ether. The ether layer was washed with water, 1N Na$_2$CO$_3$, brine and dried (MgSO$_4$), filtered and

20   concentrated. Crystallization from MeOH gave the desired epoxide-methyl ester. The methyl ester was hydrolized in refluxing methanolic KOH followed by acidification (1N HCl) to give the crude epoxide-acid **47** which was purified by crystallization from EtOAc-hex to give 600 mg (1.64 mmol) of a white powder (59% yield). MP:

25   168 °C; ^{1}H-NMR (CDCl$_3$) δ1.26 (s,CH$_3$), 1.27 (s,CH$_3$), 1.30 (s,CH$_3$), 1.31 (s,CH$_3$), 1.69 (s,(2CH$_2$)), 2.14 (s,CH$_3$), 3.15 (d,J=5.6 Hz,CH-O), 3.41 (d,J=5.6 Hz,CH-O), 7.09 (s,Ar-CH), 7.28 (d,J=8.3 Hz, Ar-2(CH)), 7.32 (s,Ar-CH), 8.01 (d,J=8.3 Hz,Ar-2(CH)).

Example 17

Preparation of compound **48** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
taken together are CH_2-CH_2(cyclopropyl), and X = COOH **(TPNCB)**:

The compound was prepared from compound **6** where R_1, R_2,

5   R_3, R_4, R_5 are methyl. To a <u>dry</u> 100 mL three necked round bottom
flask fitted with a reflux condensor, dropping funnel, and magnetic
stir bar was added 722 mg (11.65 mmol) of zinc dust, 109 mg (1.105
mmol) of cuprous chloride (CuCl), 7.5 mL of dry THF, and 1.48 g
(5.52 mmol) of diiodomethane. To the addition funnel is added 1g

10   (2.76 mmol) of compound **6** in 5 mL of dry THF. The flask is heated
to 80 °C, followed by dropwise addition of **6**. After the addition
of **6** was complete, the reaction was allowed to reflux for 30 h or
until completion, followed by dilution with 50 mL of ether and 20
mL of saturated aqueous ammonium chloride solution. The organic

15   layer was washed with 10% NaOH (3 x 20 mL), brine and dried over
anhydrous $MgSO_4$. The product was concentrated and purified by
preparative TLC (2% EtOAc-hexane) to give 220 mg (0.59 mmol) of the
methyl ester of **48**. Hydrolysis of the methyl ester with refluxing
methanolic KOH, followed by acidification (1N HCl), gave 150 mg

20   (0.41 mmol) of the desired compound **48** after crystallization from
EtOAc-hexane (15% yield). MP: 244 °C; ^{1}H-NMR ($CDCl_3$) δ1.28 (s,4
(CH_3)), 1.39 (s,CH_2-CH_2), 1.69 (s,2(CH_2)), 2.12 (s,CH_3), 6.98
(d,J=8.4 Hz,Ar-2(CH)), 7.06 (s,Ar-CH), 7.29 (s,Ar-CH), 7.91
(d,J=8.4 Hz,Ar-2(CH)).

25   **Example 18**

Preparation of compound **49** where R_1, R_2, R_3, R_4, R_5 are methyl, R' = H
and R" = CH_3, and X = COOH **(PTNEB)**:

The compound was prepared from compound **7** where R_1, R_2,
R_3, R_4, R_5 are methyl. To 1g (2.87 mmol) of compound **7** in 25 mL of

30   EtOAc was added 10 mg of 10% Pd/C. The mixture was degassed under

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vacuum followed by addition of H_2, and allowed to stir under H_2 for 2 h. The reaction was filtered through celite and the product crystallized from EtOAc-hexane to give 750 mg (2.14 mmol) of the desired product **49** (75% yield). MP: 208 °C; ^{1}H-NMR (CDCl$_3$) δ1.24

5   (s,CH$_3$), 1.25 (s,CH$_3$), 1.26 (s,CH$_3$), 1.29 (s,CH$_3$), 1.61 (d,J=7.2 Hz,CH$_3$), 1.67 (s,2(CH$_2$)), 2.12 (s,CH$_3$), 4.30(q,J=7.2 Hz, CH), 7.02 (s,Ar-CH), 7.20 (s,Ar-CH), 7.24 (d,J=8.4 Hz, Ar-2(CH)), 7.99 (d,J=8.4 Hz,Ar-2(CH)).

Example 19

10   Preparation of compound **50** where R_1, R_2, R_3, R_4, R_5 are methyl, R′ and R″ = methylidene cyclopentane, and X = COOH (**PTNCB**):

The compound was prepared from compound **4** and where R_1, R_2, R_3, R_4, R_5 are methyl. To 1g (2.87 mmol) of **4** in 25 mL of THF at 0 °C was added 8.6 mL of a 1M cyclopentenyl magnesium chloride

15   solution (8.6 mmol). After stirring for 30 m, water was added and acidified with 5 N HCl. The acidified mixture was heated for 5 m, cooled, and the organic product extracted with EtOAc. The EtOAc layer was washed with water and brine, dried (MgSO$_4$), filtered, and concentrated to give the crude product. Crystallization from EtOAc-

20   hexane gave 340 mg (0.85 mmol) of **50** as a white powder (30% yield). MP: 201 °C; ^{1}H-NMR (CDCl$_3$) δ1.27 (s,4(CH$_3$)), 1.64 (br t,CH$_2$), 1.68 (s,2(CH$_2$)), 1.70 (br t,CH$_2$), 1.97 (s,CH$_3$), 2.15 (br t,CH$_2$), 2.56 (br t,CH$_2$), 7.04 (s,Ar-CH), 7.05 (s,Ar-CH), 7.29 (d,J=8 Hz,Ar-2(CH)), 7.97 (d,J=8 Hz,Ar-2(CH)).

Example 20

25   Preparation of compound **51** where R_1, R_2, R_3, R_4, R_5 are methyl, R′ and R″ = isopropylidene, and X = COOH (**PTNIB**):

The compound was prepared from compound **4** and where R_1, R_2, R_3, R_4, R_5 are methyl. To 1g (2.87 mmol) of **4** in 25 mL of THF at

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0°C was added 8.6 mL of a 1M isopropyl magnesium chloride solution (8.6 mmol). After stirring for 30 m, water was added and acidified with 5 N HCl. The acidified mixture was heated for 5 m, cooled, and the organic product extracted with EtOAc. The EtOAc layer was

5   washed with water and brine, dried (MgSO$_4$), filtered, and concentrated to give the crude isopropylidene product. Crystallization from EtOAc-hexane gave 550 mg (1.46 mmol) of **51** as a white powder (51% yield). MP: 297°C; ^{1}H-NMR (CDCl$_3$) δ1.25 (br s,4(CH$_3$)), 1.64 (s,=CCH$_3$), 1.66 (s,=CCH$_3$) 1.87 (s,2(CH$_2$)), 1.96

10   (s,CH$_3$), 7.00 (s,Ar-CH), 7.03 (s,Ar-CH), 7.25 (d,J=8 Hz,Ar-2(CH)), 7.97 (d,J=8 Hz,Ar-2(CH)).

Example 21

Preparation of compound **52** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R" = oxo, Z = S, and X = COOH **(TTNCTC)**:

15   To 1 g (4.9 mmol) of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydro-naphthalene and 1 g (4.9 mmol) of mono methyl thiophene carboxylic acid chloride in 25 mL of CH$_2$Cl$_2$ was added 1 g (7.5 mmol) of AlCl$_3$. The reaction was heated to reflux for 15 m followed by cooling and addition of 20% aqueous HCl. The product was extracted

20   with EtOAc, washed (H$_2$O, brine), dried (MgSO$_4$), filtered, concentrated, and purified by crystallization from MeOH to give 450 mg (1.21 mmol) of the methyl ester of **52** (25% yield). The methyl ester was hydrolized in methanolic KOH followed by acidification (20% HCl) extraction with EtOAc, washed (H$_2$O, brine), dried (MgSO$_4$),

25   filtered, concentrated, and purified by crystallization from EtOAc-hexane to give 375 mg (1.05 mmol) of **52** (87% yield). MP: 206 °C; ^{1}H-NMR (CDCl$_3$) δ1.26 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.71 (s,2(CH$_2$)), 2.38 (s,CH$_3$), 7.21 (s,Ar-CH), 7.44 (s,Ar-CH), 7.48 (d,J=4 Hz, Thio Ar-CH), 7.85 (d,J=4 Hz,Thio Ar-CH).

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Example 22

Preparation of compound **53** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
= methano, Z = S, and X = COOH **(TTNETC)**:

5   Compound **53** was prepared from the methyl ester of **52** in a
manner similar to examples 4 and 5. MP: 200 °C; ^{1}H-NMR (CDCl$_3$)
δ1.26 (s,2(CH$_3$)), 1.30 (s,2(CH$_3$)), 1.69 (s,2(CH$_2$)), 2.10 (s,CH$_3$),
5.21 (s,=CH), 5.88 (s,=CH), 6.76 (d,J=4 Hz,Thio Ar-CH), 7.11 (s,Ar-
CH), 7.23 (s,Ar-CH), 7.68 (d,J=4 Hz,Thio Ar-CH).

Example 23

10 Preparation of compound **54** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
= oxo, and X = tetrazole **(3-methyl-TTNCBT)**:

To 500 mg (1.51 mmol) of 4-[(3,5,5,8,8-pentamethyl-
5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzonitrile (synthesized by
AlCl$_3$ catalyzed condensation of 1,1,4,4,6-pentamethyl-1,2,3,4-tetra
15 hydronaphthalene with 4-cyanobenzoic acid chloride in CH$_2$Cl$_2$) in
toluene was added 342 mg (1.66 mmol) of trimethyl tin azide. The
mixture was refluxed for 23 h and cooled to give 537 mg (1.44 mmol)
of the desired tetrazole **54** as a white precipitate (96% yield).
LRMS: 374.15; ^{1}H-NMR (CD$_3$SOCD$_3$) δ1.19 (s,2(CH$_3$)), 1.32 (s,2(CH$_3$)),
20 1.70 (s,2(CH$_2$)), 2.25 (s,CH$_3$), 3.19 (s,N-H), 7.30 (s,Ar-CH), 7.32
(s,Ar-CH), 7.90 (d,J=8 Hz,Ar-2(CH)), 8.20 (d,J=8 Hz,Ar-2(CH)).

Example 24

Preparation of compound **55** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
= methano, and X = tetrazole **(3-methyl-TTNEBT)**:

25 To 500 mg (1.52 mmol) of 4-[1-(3,5,5,8,8-pentamethyl-
5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzonitrile (synthesized by
AlCl$_3$ catalyzed condensation of 1,1,4,4,6-pentamethyl-1,2,3,4-
tetrahydronaphthalene with 4-cyanobenzoic acid chloride in CH$_2$Cl$_2$
followed by treatment of the ketone with CH$_3$PPh$_3$Br-NaNH$_2$) in toluene

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was added 342 mg (1.67 mmol) of trimethyl tin azide. The mixture
was refluxed for 23 h and cooled to give 535 mg (1.44 mmol) of the
desired tetrazole **55** as a white precipitate (95% yield). LRMS:
372.25; ^{1}H-NMR (CD_3SOCD_3) δ1.21 (s,2(CH_3)), 1.24 (s,2(CH_3)), 1.68

5   (s,2(CH_2)), 1.92 (s,CH_3), 2.55 (s,N-H), 5.27 (=CH), 5.97 (s,=CH),
7.10 (s,Ar-CH), 7.18 (s,Ar-CH), 7.47 (d,J=8 Hz,Ar-2(CH)), 8.00
(d,J=8 Hz,Ar-2(CH)).

Example 25

Preparation of compound **25** where R_1,R_2,R_3,R_4 are methyl, R' and R" =

10   oxo, and X = COOMe:

The compound was prepared in a manner similar to that of
compound **4** except that 1,1,4,4-tetramethyl-1,2,3,4-
tetrahydronaphthalene was substituted for 1,1,4,4,6-pentamethyl-
1,2,3,4-tetrahydronaphthalene and 4-methyl ester pyridinic 2-acid

15   chloride was substituted for mono-methyl terephthalic acid chloride
(see examples 1 and 2).

Example 26

Preparation of compound **56** where R_1,R_2,R_3,R_4 are methyl, R' and R" =
methano, and X = COOH **(TTNEP)**:

20   Compound **25** was treated with CH_3PPh_3Br-$NaNH_2$ as in example
#4. Hydrolysis of the resulting olefinic methyl ester with
methanolic KOH, followed by acidification (20% HCl) and
crystallization from EtOAc-hexane gave compound **56**. MP: 173 °C;
^{1}H-NMR ($CDCl_3$) δ1.26 (s,(CH_3)), 1.27 (s,CH_3), 1.30 (s,2(CH_3)), 1.70

25   (s,(CH_2)), 5.70 (s,=CH), 6.10 (s,=CH), 7.08 (d,J=8 Hz,Pyr-CH), 7.27
(s,Ar-CH), 7.19 (d,J=8 Hz,Ar-CH), 7.39 (d,J=8 Hz,Ar-CH), 8.28
(d,J=8 Hz,Pyr-CH), 9.31 (s,Pyr-CH).

Example 27

Preparation of compound **57** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
= oxo, and X = COOH:

5   Compound **57** was prepared in a manner similar to that of
compound **6** (example #4) except that 4-methylester-pyridinic-2-acid
chloride was substituted for mono-methyl terephthalic acid chloride
(see examples 1 and 2). The resulting methyl ester was hydrolyzed
as in example #5 to give compound **57**. ^{1}H-NMR (CDCl$_3$) δ1.22

10   (s,2(CH$_3$)), 1.30 (s, 2(CH$_3$)), 1.69 (s,2(CH$_2$)), 2.40 (s,CH$_3$), 7.22
(s,Ar-CH), 7.43 (s,Ar-CH), 8.13 (d,J=8.0 Hz,Pyr-CH), 8.54 (d,J=8
Hz,Pyr-CH), 9.34 (s,Pyr-CH).

Example 28

Preparation of compound **58** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R"
= methano, and X = COOH **(TPNEP)**:

15   The methyl ester from example #26 was treated with
CH$_3$PPh$_3$Br-NaNH$_2$ as in example #4 followed by hydrolysis with
methanolic KOH at reflux for 1 h and acidification with 20% aqueous
HCl and crystallization from EtOAc-hexane to give compound **58**. MP:
235 °C; ^{1}H-NMR (CDCl$_3$) δ1.27 (s,2(CH$_2$)), 1.31 (s,2(CH$_3$)), 1.70

20   (s,2(CH$_2$)), 2.00 (s,CH$_3$), 5.55 (s,=CH), 6.57 (s,=CH), 7.06 (d,J=8.3
Hz,Pyr-CH), 7.12 (s,Ar-CH), 7.14 (s,Ar-CH), 8.20 (d,J=8.1 Hz,Pyr-
CH). 9.29 (s,Pyr-CH).

Example 29

Preparation of methyl 2-acetyl-5-pyridinecarboxylate **32**:

25   To a slurry of the 2,5-pyridinedicarboxylic acid **29** (34
g, 0.2 mol) in 120 mL of methanol at 0°C was added dropwise 15 mL
of thionyl chloride and the resulting slurry was warmed up to room
temperature, giving rise to a clear solution. The mixture then was
heated at reflux for 12h and to afford a yellow slurry. Filtration

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of the reaction mixture provided dimethyl-2,5-pyridinedicarboxylate **30** in quantitative yield as a yellow crystalline solid.

The pyridinedicarboxylate **30** (19.5 g, 0.1 mol) was treated with solid KOH (6.51 g, 0.1 mol) in 300 mL of methanol at

5   room temperature for 2 h, giving rise to a thick pale white suspension, which was filtered and dried to provide the mono-potassium pyridinecarboxylate **3** in quantitative yield.

The crude mono-pyridinecarboxylate **31** (880 mg, 4 mmol) was treated with 3 mL of thionyl chloride at reflux for 2h and the

10   excess $SOCl_2$ was removed by the usual method. To the crude acid chloride in 8 mL of THF at -78°C was added slowly a freshly prepared 1.0M ether solution (5.5 mL, 5.5 mmol) Me_2CuLi. The resulting dark slurry was allowed to stir at -78 °C for 60 min. and then was quenched with 2% HCl. Standard work-up and chromatography

15   of the crude mixture afforded methyl-2-acetyl-5-pyridinecarboxylate **32** in over 56% yield as a yellow solid.

Example 30

Preparation of compound **58** **(TPNEP)** (by an alternate scheme than in Example 28) and of corresponding ester **Et-58** where R_1,R_2,R_3,R_4,R_5 are

20   methyl, R' and R" are methano, and = COOH:

A solution of 2-bromotoluene (8.5 g, 50 mmol) and 2,2-dichloro-2,2-dimethylhexane (9.15 g, 50 mmol) in 100 mL of dichloroethane was treated with aluminum trichloride (0.66 g, 5 mmol). The resulting dark brown solution was allowed to stir at

25   room temperature for 30 min. and was then quenched with ice. Removal of solvent and recrystallization from methanol afforded 2-bromo-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene **33** in 95% yield as a white solid. A THF (4 mL) solution containing the bromocompound **33** (141 mg, 0.5 mmol) at -78 °C was treated with a

30   1.6 M hexane solution (0.4 mL, 0.6 mmol) of n-BuLi, and the

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resulting mixture was then cannulated to a THF (2 mL) solution of
the 2-acetyl-5-pyridinecarboxylate **32** (72 mg, 0.4 mmol) at -78 °C.
The mixture was allowed to stir at -78 °C for 60 min. and was
quenched with 2% HCl. Removal of the solvent and chromatography of

5   the crude mixture provided the intermediate **34**, which was then
treated with 5% HCl at reflux followed by KOH-MeOH at 70°C for 30
min. Standard work-up and chromatography of the crude mixture
provided 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2
naphthyl)ethenyl] pyridine-5-carboxylic acid **58** in over 50% yield

10   as a white solid. ^{1}H-NMR (CDCl$_3$) δ1.27 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)),
1.70 (s,2(CH$_2$)), 2.00 (s, CH3), 5.56 (s,=CH), 6.55 (s,=CH), 7.08
(d,J = 8.3 Hz, Pyr-CH), 7.12 (s,Ar-CH), 7.15 (s,Ar-CH), 8.23 (d,J =
8.3 Hz,Pyr-CH) and 9.32 (s,Pyr-CH).

Treatment of the pyridinecarboxylic acid **58** (15 mg, 0.004

15   mmol) with one drop of SOCl$_2$ in 5 mL of ethanol at reflux for 60 m,
followed by a flash chromatography, gave rise to a quantitative
yield of the ethyl ester **Et-58** as a white solid. ^{1}H-NMR (CDCl$_3$)
δ1.27 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.40 (t,J = 7.1 Hz,
-CH$_2$CH$_3$), 1.70 (s,2(CH$_2$)), 1.99 (s,CH$_3$), 4.40(q,J = 7.1 Hz, -CH$_2$CH$_3$),

20   5.51 (s,=CH), 6.53 (s,=CH), 7.01 (d, J = 8.0 Hz,Pyr-CH), 7.12
(s,Ar-CH), 7.14 (s,Ar-CH), 8.15 (d,J = 8.0 Hz,Pyr-CH) and 9.23
(s,Pyr-CH).

Example 31

Preparation of 3-acetyl-2-pyridinecarboxylic acid N,N-diisopro-
25   pylamide **36a**:

The mono-potassium pyridinecarboxylate **31** (1.1 g, 5 mmol)
was treated with SOCl$_2$ (5 mL, excess) at 70 °C for 2h and the
excess thionyl chloride was removed to give a yellowish solid. To a
solution of diisopropylamine (1 g, 10 mmol) in 10 mL of methylene
30   chloride at 0 °C was added the CH$_2$Cl$_2$ solution (10 mL) of the above

50

acid chloride. The resulting slurry was allowed to stir at room
temperature for 3 h and was filtered from the ammonium salts.
Removal of solvent and chromatography of the crude residue afforded
the product **36a** in 90% yield as a white solid.

5 **Example 32**

Preparation of compounds **60 (TPNEPC)** and **61 (3TTNEPE)**:

2-Bromo-3,5,5,8,8-pentamethyl-5,6,7,8-
tetrahydronaphthalene **33** (620 mg, 2.2 mmol) and the
acetylpyridineamide **36a** (500 mg, 2 mmol) were converted by a
10 similar method as described above to obtain the intermediate **34** in
over 80% yield. To a solution of the pyridine amide **34** (432 mg, 1
mmol) in 5 mL of THF at -78°C was added 1.5 M DIBAL toluene
solution (0.7 mL, 1.05 mmol) and the resulting yellow clear
solution was warmed up to -20 °C slowly in 60 min. and then was
15 quenched with water. Removal of solvent and chromatography of the
crude mixture afforded the pyridinealdehyde **35** in 83% yield as a
white solid. ^{1}H-NMR (CDCl$_3$) δ1.25 (s,2(CH$_3$)), 1.29 (s,2(CH$_3$)), 1.70
(s,2(CH$_2$)), 1.96 (s,CH$_3$), 5.47 (s,=CH), 5.92 (s,=CH), 7.10 (s,Ar-
CH), 7.11 (s,Ar-CH), 7.70 (d,J = 8.0 Hz,Pyr-CH), 7.88 (d,J = 8.0
20 Hz,Pyr-CH), 8.72 (s, Pyr-CH), 10.06 (s,CHO).

The pyridinealdehyde **35** (10 mg, 0.03 mmol) was treated
with 2.0 mL of H$_2$O$_2$ in 2 mL of methanol-water 1:1 mixture at room
temperature for 10 h and then was quenched with 10% HCl. Extraction
of the mixture with EtOAc (40 mL) and removal of solvent gave rise
25 to 5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-
naphthyl)ethenyl]pyridine-2-carboxylic acid **60** as a white solid in
almost quantitative yield. ^{1}H-NMR (CDCl$_3$) δ1.26 (s,2(CH$_3$)), 1.30
(s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 1.94 (s,CH$_3$), 5.47 (s,=CH), 5.92
(s,=CH), 7.10 (s,Ar-2(CH)), 7.76 (bs,Pyr-CH, 8.16 (bs,Pyr-CH) and
30 8.65 (s,Pyr-CH).

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Treatment of the pyridinecarboxylic acid **60** (5 mg) with one drop of $SOCl_2$ in 1 mL of ethanol at reflux for 60 min followed by a flash chromatography gave rise to a quantitative yield of the ethyl ester **61** as a white solid. ^1H-NMR ($CDCl_3$) δ1.26 (s,2(CH_3)),

5   1.29 (s,2(CH_3)), 1.44 (t,J = 7.1 Hz,$-CH_2CH_3$), 1.69 (s,2(CH_2)), 1.95 (s,CH_3), 4.46 (q,J = 7.1 Hz,$-CH_2CH_3$)), 5.43 (s,=CH), 5.88 (s,=CH), 7.09 (s,Ar-CH), 7.10 (s,Ar-CH), 7.64 (d,J = 8.0 Hz, Pyr-CH), 8.03 (d,J = 8.0 Hz,Pyr-CH) and 8.68 (s,Pyr-CH).

Example 33

10   Preparation of compound **62** where R_1,R_2,R_3,R_4,R_5 are methyl and R' and R" together are CH_2CH_2 (**TPNCP**):

To 162 mg (2.48 mmol) of zinc dust, 25 mg (0.25 mmol) of CuCl, and 332 mg (1.24 mmol) of CH_2I_2 in 3 mL of dry ether was added dropwise 150 mg (0.413 mmol) of olefin **26** where R_1, R_2, R_3, R_4, R_5

15   are methyl in 5 mL of dry ether. The mixture was heated at reflux for 12 h or until complete by H-NMR. Water was added and the organics extracted with ether, washed with NH_4Cl, brine and dried over $MgSO_4$. The desired cyclopropyl compound was purified by crystallization from ether-MeOH to give 60 mg (0.159 mmol) of the

20   methyl ester of **62** as a pale yellow solid (39% yield). MP: 177 °C; ^1H-NMR ($CDCl_3$) δ1.27 (s,2(CH_3)), 1.31 (s,2(CH_3)), 1.35 (s,CH_2), 1.70 (s,2(CH_2)), 1.85 (s,CH_2), 2.11 (s,CH_3), 3.90 (s,CH_3), 6.75 (d,J = 8.0 Hz,Pyr-CH), 7.14 (s,Ar-CH), 7.26 (s,Ar-CH), 7.98 (d,J = 8.0 Hz,Pyr-CH) and 9.23 (s,Pyr-CH).

25   To 60 mg (0.16 mmol) of the above methyl ester in 10 mL of MeOH was added 1 mL of an aqueous 6N KOH solution. After stirring at room temperature for 1 h, the hydrolysis was complete and the reaction was acidified with 1 N aqueous HCl until the solids precipitated. The product was extracted with ether, washed

30   with water, brine and dried over $MgSO_4$. Crystallization from EtOAc-

hexanes gave 33 mg (0.094 mmol) of the pyridinal carboxylic acid **62** (59% yield). MP: 275 °C; ^{1}H-NMR(CDCl$_3$) δ1.25 (s,2(CH$_3$)), 1.35 (s,2(CH$_3$)), 1.40 (s,CH$_2$), 1.72 (s,2(CH$_2$)), 1.85 (s,CH$_2$), 2.15(s,CH$_3$), 6.78 (d,J = 8.0 Hz,Pyr-CH), 7.14 (s,Ar-CH), 7.26 (s,Ar-CH), 8.02

5 (d,J = 8.0 Hz,Pyr-CH) and 9.15 (s,Pyr-CH).

Example 34

Preparation of compound **63** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R" are methano, X = CONHR$_9$, and R$_9$ = 4-hydroxyphenyl (**3-methyl-TTNEHBP**):

10 To 750 mg (10 mml) of DMF in 22 mL of anhydrous ether was added 1.3g (10 mmol) of oxalyl chloride. The reaction was stirred for 1 h, followed by removal of solvent to give a crude white solid (dimethylchloroformadinium chloride). To the dimethylchloro-formadinium chloride was added 2.87 g (8.24 mmol) of compound **7** in

15 12 mL of dry DMF. The reaction was stirred for 20 m at room temperature followed by cooling to 0°C. The cooled solution of the acid chloride of **7** was added dropwise to a cooled DMF (0°C) solution containing 3.62 g (33 mmol) of 4-aminophenol and 1.68 g (16.3 mmol) of triethyl amine. After stirring at 0°C for 30 m, the

20 reaction was warmed to room temperature for 12 h. Aqueous 20% HCl was added and the resulting solid was filtered and washed with water, acetone, and EtOAc to give 600 mg (1.36 mmol) of the desired compound **63** (17% yield). ^{1}H-NMR (CDCl$_3$) δ1.29 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.71 (s,(CH$_2$)), 1.99 (s,CH$_3$), 5.31 (s,=CH), 5.80

25 (s,=CH), 6.85 (d,Ar-2(CH)), 7.09 (s,Ar-CH), 7.16 (s,Ar-CH), 7.40 (d,Ar-2(CH)), 7.48 (d,Ar-2(CH)), 8.40 (d,Ar-2(CH)).

Example 35

Preparation of compound **64** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R" are methano, X = CONHR$_9$, and R$_9$ = 4-fluorophenyl (**3-methyl-TTNEFBP**):

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The compound was prepared in a manner similar to that of compound **63** except that 4-fluoroaniline was substituted for 4-aminophenol. MP: 203°C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 1.96 (s,CH$_3$), 5.33 (s,=CH), 5.81

5 (s,=CH), 7.05 (d,J=9 Hz),Ar-2(CH)), 7.09 (s,Ar-CH), 7.13 (s,Ar-CH), 7.39 (d,J=8.4 Hz,Ar-2(CH)), 7.59 (dd,J=5,9 Hz,Ar-2CH), 7.75 (brs NH), 7.78 (d,J=8.4 Hz,Ar-2(CH)).

Example 36

Preparation of compound **65** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R"

10 are methano, X = CONHR$_9$, and R$_9$ = 4-phenylcarboxylic acid **(3-methyl-TTNECBP)**:

The compound was prepared in a manner similar to that of compound **63** except that methyl 4-aminophenyl carboxylate was substituted for 4-aminophenol. The resulting ester was hydrolyzed

15 in methanolic KOH, followed by acidification (20% HCl) to give the desired compound **65**. MP: 200 °C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)), 1.31 (s,2(CH$_3$)), 1.71 (s,2(CH$_2$)), 1.97 (s,CH$_3$), 5.34 (s,=CH), 5.85 (s,=CH), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.40 (d,J=8 Hz,Ar-CH), 7.80 (d,J=8 Hz,Ar-2(CH)), 7.87 (br s,Ar-2(CH)), 8.14 (br s,Ar-

20 2(CH)).

Example 37

Preparation of compound **66** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R" are oxo, X = CONHR$_9$, and R$_9$ = 3-hydroxyphenyl **(3-methyl-m-TTNCHBP)**:

To 750 mg (10 mml) of DMF in 22 mL of anhydrous ether was

25 added 1.3g (10 mmol) of oxalyl chloride. The reaction was stirred for 1 h, followed by removal of solvent to give a crude white solid (dimethylchloroformadinium chloride). To the dimethylchloroformadinium chloride was added 2.88 g (8.24 mmol) of compound **4** in 12 mL of dry DMF. The reaction was stirred for 20 m at room

temperature, followed by cooling to 0°C. The cooled solution of the

acid chloride of **7** was added dropwise to a cooled DMF (0°C)

solution containing 3.62 g (33 mmol) of 4-aminophenol and 1.68 g

(16.3 mmol) of triethyl amine. After stirring at 0°C for 30 m, the

5   reaction was warmed to room temperature for 12 h. Aqueous 20% HCl

was added and the resulting solid was filtered and washed with

water, acetone, and EtOAc to give 750 mg (1.70 mmol) of the desired

compound **66** (21% yield). MP: 182°C; ^{1}H-NMR (CDCl$_3$) δ1.22

(s,2(CH$_3$)), 1.32 (s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 2.37 (s,CH$_3$), 6.58

10 (m,Ar-2(CH)), 7.20 (d,J=8 Hz,Ar-CH), 7.22 (s,Ar-CH), 7.28 (s,Ar-

Ch), 7.91 (d,J=8.3 Hz,Ar-2(CH)), 8.26 (d,J=8.3 Hz,Ar-2(CH)).

Example 38

Preparation of compound **67** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R"

are methano, X = CONHR$_9$, and R$_9$ = 3-hydroxyphenyl **(3-methyl-m-**

15 **TTNEHBP):**

The compound was prepared in a manner similar to that of

compound **63** except that 3-aminophenol was substituted for 4-

aminophenol. MP: 136°C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)), 1.31

(s,2(CH$_3$)), 1.70 (s,2(CH$_2$)), 1.97 (s,CH$_3$), 5.35 (s,=CH), 5.84

20 (s,=CH), 6.57 (m,Ar-2(CH)), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.16

(m,Ar-CH), 7.39 (d,J=8.3 Hz,Ar-2(CH)), 8.09 (d,J=8.3 Hz,Ar-2(CH)).

Example 39

Preparation of compound **68** where R$_1$,R$_2$,R$_3$,R$_4$,R$_5$ are methyl, R' and R"

are methano, X = CONHR$_9$, and R$_9$ = 2-hydroxyphenyl **(3-methyl-o-**

25 **TTNCHBP):**

The compound was prepared in a manner similar to that of

compound **63** except that 2-aminophenol was substituted for 4-

aminophenol. MP: 180°C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)), 1.31

(s,2(CH$_3$)), 1.71 (s,2(CH$_2$)), 1.97 (s,CH$_3$), 5.35 (s,=CH), 5.84

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(s,=CH), 6.9 (m,Ar-CH), 7.08-7.2 (m,Ar-CH), 7.09 (s,Ar-CH), 7.13

(s,Ar-CH), 7.42 (d,J=8.4 Hz,Ar-2(CH)), 7.83 (d,J=8.4 Hz,Ar-2(CH)),

8.03 (brs,Ar-CH), 8.64 (s,NH).

Example 40

5   Preparation of compound **69** where R_1,R_2,R_3,R_4,R_5 are methyl, R' and

R" are methano, X = $CONHR_9$, and R_9 = 3-phenylcarboxylic acid **(3-
methyl-m-TTNECBP)**:

The compound was prepared in a manner similar to that of

compound **63** except that methyl-3-amino phenyl carboxylate was

10   substituted for 4-aminophenol. The resulting ester was hydrolyzed

in methanolic KOH followed by acidification (20% HCl) to give the

desired compound **69**. MP: 250°C; ^{1}H-NMR (CDCl$_3$) δ1.28 (s,2(CH$_3$)),

1.31 (s,2(CH$_3$)), 1.71 (s,2(CH$_2$)), 1.97 (s,CH$_3$), 5.34 (s,=CH), 5.85

(s,=CH), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.40 (d,J=8 Hz, Ar-2(CH)),

15   7.55 (m,Ar-CH),7.76 (m,Ar-CH), 7.80 (d,J=8 Hz, Ar-2(CH)), 7.87

(s,Ar-CH), 8.14 (s,NH).

Example 41

Preparation of compound **70** where R_1,R_2,R_3,R_4,R_5 are methyl, R' and R"

are methano, n=0, and X = COOH:

20   The compound was prepared in a manner similar to that of

compound **7** except that 1,1,3,3,5-pentamethylindane was substituted

for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples

1, 2, 4, and 5. MP: 145°C; ^{1}H-NMR (CDCl$_3$) δ1.05 (s,2(CH$_3$)), 1.28

(s,CH$_3$), 1.31 (s,CH$_3$), 1.38 (s,CH$_2$), 1.98 (s,CH$_3$), 5.34 (s,CH), 5.84

25   (s,CH), 6.90 (s, Ar-CH), 6.92 (s, Ar-CH), 7.36 (d,J=8.4 Hz, Ar-2

(CH)), 8.00 (d,J=8.4 Hz,Ar-2(CH)).

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Example 42

Preparation of compound **71** where $R_1, R_2, R_3, R_4, R_5, R_{14}$ are methyl, R' and R" are methano, n=0, and X = COOH:

The compound was prepared in a manner similar to that of
5   compound **7** except that 1,1,2,3,3,5-pentamethylindane was

substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene

in examples 1, 2, 4, and 5. MP: 217°C; ^{1}H-NMR ($CDCl_3$) δ1.01

$(d,J=7.3 Hz,CH_3)$, 1.08 (s,CH_3), 1.10 (s,CH_3), 1.27 (s,CH_3), 1.30

(s,CH_3), 1.88 (q,CH), 2.00 (s,CH_3), 5.35 (s,=CH), 5.85 (s,=CH), 6.95
10   (s,Ar-CH), 6.98 (s,Ar-CH), 7.38(d,J=8.3 Hz,Ar-2(CH)), 8.00 (d,J=8.3

Hz,Ar-2(CH)).

Example 43

Preparation of compound **72** where R_1, R_2, R_3, R_4, R_5, are methyl, R' and
R" are H, and X = COOH:

15   The compound was prepared in a manner similar to that of

compound **4** (examples 1 and 2) except that methyl-4-

(bromomethyl)benzoate was substituted for mono-methyl terephthalic

acid chloride. MP: 237°C; ^{1}H-NMR ($CDCl_3$) δ1.23 $(s,2(CH_3))$, 1.27

$(s,2(CH_3))$, 1.67 $(s,2(CH_2))$, 2.16 (s,CH_3), 4.06 (s,CH_2), 7.01 (s,Ar-
20   CH), 7.08 (s,Ar-CH), 7.25 (d,J=8 Hz,Ar-2(CH)), 8.01 (d,J=8 Hz,Ar-

2(CH)).

Example 44

Preparation of compound **111** where R_1, R_2, R_3, R_4, R_5 are methyl and R'
and R" together are CH_2CH_2:

25   To 200 mg (0.573 mmol) of ester **25** in 10 mL of dry dichloro

ethane under dry nitrogen at 0°C was added 0.29 mL (2.87 mM) of

Et_2Zn. To this solution was added $ClCH_2I$ dropwise via a syringe and

the reaction mixture was stirred by 0°C for 10 m. The solution was

57

then warmed to 55°C for 6 h or until complete by TLC. Water was added and the organics extracted with ether, washed with NH_4Cl, brine and dried over $MgSO_4$. The desired cyclopropyl compound was purified by SiO_2 column chromatrography to give 30 mg (0.083 mmol)

5   of the methyl ester of **73** as a white solid (14% yield). MP: 160°C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 2(CH_3)), 1.3 (s, 2(CH_3)), 1.35 (s, CH_2)), 1.70 (s, 2(CH_2)), 1.72 (s, CH_2), 3.90 (s, OCH_3), 6.87 (d, J = 8.0 Hz, Pyr-CH), 7.12 (d, J = 8 Hz, Ar-CH), 7.27 (s, Ar-CH), 7.32 (d, J = 8 Hz, Ar-CH), 7.98 (d, J = 8.0 Hz, Pyr-CH) and 9.08 (s, Pyr-CH).

10   To 30 mg (0.083 mmol) of the above methyl ester in 5 mL of MeOH was added 1 mL of an aqueous 6N KOH solution. After stirring at RT for 1 h, the hydrolysis was complete and the reaction was acidified with 1 N aqueous HCl until the solids precipitated. The product was extracted with ether, washed with water, brine and

15   dried over $MgSO_4$. Crystallization from EtOAc-hexanes gave 18 mg (0.051 mmol) of the pyridinal carboxylic acid **111** (62% yield). MP: 255°C; ^{1}H-NMR($CDCl_3$) δ 1.25 (s, 2(CH_3)), 1.31 (s, 2(CH_3)), 1.40 (s, CH_2), 1.72 (s, 2(CH_2)), 1.75 (s, CH_2), 6.89 (d, J = 8.0 Hz, Pyr-CH), 7.12 (d, J = 8 Hz, Ar-CH), 7.27 (s, Ar-CH), 7.30 (d, J = 8 Hz, Ar-

20   CH), 8.02 (d, J = 8.0 Hz, Pyr-CH), and 9.11 (s, Pyr-CH).

Example 45

Preparation of compound **112**, where R_1, R_2, R_3, R_4, R_5 are methyl; R' and R" together are oxime (HO-N=); X = COOH **(oxime of 3-methyl-TTNCB)**:

25   3-methyl-TTNCB (4.41g, 12.6 mmol) in EtOH (10 mL) and pyridine (15.3 mL) was treated with hydroxylamine hydrochloride (4.38 g, 63 mmol), and the mixture was heated at reflux. After 6 h, the

mixture was cooled to room temperature and the ethanol was removed *in vacuo*. The residue was taken-up in water and the aqueous layer was adjusted to pH = 4-5 with 1 M aqueous HCl. The aqueous solution was extracted 3 times with EtOAc; the organic layers were combined,

5   and washed with water (2x) and brine. The organic solution was dried (NaSO$_4$), filtered, and concentrated to give a foamy white solid. Recrystallization (CH$_2$Cl$_2$/ether/ hexanes) gave a white solid, 4.05 g (88%). MP: 204-209°C(d); HRMS: 366.2060 (MH+);^{1}H-NMR (CDCl$_3$/d-4 MeOH) δ 1.22 (s, 6H, 2 (CH$_3$)), 1.32 (s, 6H, 2(CH$_3$)),

10   1.69 (s, 4H, 2(CH$_2$)), 2.11 (s, 3H, CH$_3$,), 6.99 (s, 1H, Ar-CH), 7.20 (s, 1H, Ar-CH), 7.53 (½ABq, 2H, J = 8.4 Hz, $\Delta\upsilon$ = 183.0 Hz, Ar-CH), 7.99 (½ ABq, 2H, J = 8.4 Hz, $\Delta\upsilon$ = 183.0 Hz-Ar-CH).

Example 46

Preparation of compound **113**, where R$_1$, R$_2$, R$_3$, R$_4$ are methyl; R$_5$ is

15   bromo; R' and R" are oxime (HO-N=); X = COOH **(oxime of 3-bromo-TTNCB, 41)**:

    3-bromo-TTNCB methyl ester (399 mg, 0.93 mmol) in MeOH (2 mL) was treated with hydroxylamine hydrochloride (97 mg. 1.4 mmol) and KOH (156 mg, 2.8 mmol), and the mixture was heated at reflux for 3

20   h. The reaction was worked-up in a manner identical to that described for **112** to give a white solid 330 mg (83%). MP: 240-244°C(d); ^{1}H-NMR (CDCl$_3$) δ 1.26 (s, 6H, 2(CH$_3$)), 1.33 (s, 6H, 2(CH$_3$)), 1.72 (s, 4H, 2(CH$_2$)), 7.30 (s, 1H, Ar-CH), 7.54 (s, 1H, Ar-CH), 7.92 (½ABq, 2H, J = 8.3 Hz $\Delta\upsilon$ = 113.5 Hz, Ar-CH), 8.20 (½ABq,

25   2H, J = 8.3 Hz $\Delta\upsilon$ = 113.5 Hz, Ar-CH).

Example 47

Preparation of compound **114**, where R_1, R_2, R_3, R_4, R_5 are methyl; R'
and R" are oxime (HO-N=); X = COOH; Z = N; Z', Z", Z'" = CH (**oxime
of 57**):

5   The compound was prepared in a manner similar to that
described for **113**, except compound **57**, rather than 3-methyl-TTNCB,
was the starting ketone. Flash chromatography on SiO_2,
(hexanes:EtOAc:CH_2Cl_2:isopropanol = 10:5:1:1) of the crude product,
gave a sticky, white solid, 83 mg (86%). MP: 167-172°C(d); HRMS:
10   367.2025(MH^+); FTIR (neat) 3600-3230 (br), 2962, 2928, 2664, 1697,
1593, 1296, 1273, 1122, 1028, 964 cm^{-1},^{1}H-NMR ($CDCl_3$), δ 1.23 (s, 6H,
2 (CH_3)), 1.32 (s, 6H, 2(CH_3)), 169 (s, 4H, 2 (CH_2)), 2.14 (s, 3H,
CH_3), 7.05 (s, 1H, Ar-CH), 7.22 (s, 1H, Ar-CH), 7.49 (½ABq, 2H, J =
28.0 Hz, Ar-CH), 8.25 (½ABq, 2H, J= 8.0 Hz, Ar-CH); 9.21 (s, 1H,
15   Ar-CH).

Example 48

Preparation of compound **115**, where R_1, R_2, R_3, R_4, R_5 are methyl; R'
and R" are methoxyoxime (CH_3O-N=); X = COOH (**methoxyoxime of 3-
methyl-TTNCB**):

20   3-methyl-TTNCB (560 mg, 1.60 mmol) in EtOH (2 mL) and pyridine
(1.3 mL) was treated with methoxylamine hydrochloride (402 g, 4.81
mmol), and the mixture was heated at reflux. After 6 h, the
mixture was cooled to room temperature and the ethanol was removed
in vacuo. The residue was taken-up in water and the aqueous layer was
25   adjusted to pH = 4-5 with 1 M aqueous HCl. The aqueous solution
was extracted 3 times with EtOAc, the organic layers were combined,
and washed with water (2x) and brine. The organic solution was

dried (NaSO$_4$), filtered, concentrated, and crystallized (CH$_2$Cl$_2$/hexanes) to give a white solid, 564 mg (93%). MP:228-228.5°C; LRMS: 380 (MH$^+$); ^{1}H-NMR (CDCl$_3$) δ 1.22 (s, 6H, 2 (CH$_3$)), 1.31 (s, 6H, 2 (CH$_3$)), 1.69 (s, 4H, 2(CH$_2$)), 2.08 (s, 3H, CH$_3$), 4.01

5   (s, 3H, OCH$_3$), 6.95 (s, 1H, Ar-CH), 7.18 (s, 1H, Ar-CH), 7.57 ($\frac{1}{2}$ABq, 2H, J = 8.4 Hz, $\Delta\upsilon$ = 188.3 Hz, Ar-CH), 8.04 ($\frac{1}{2}$ABq, 2H, J = 8.4 Hz, $\Delta\upsilon$ = 188.3 Hz, Ar-CH).

Example 49

Preparation of compound **116**, where R1, R2, R3, R4, R5 are methyl;

10   R' and R" are methyloxime (CH$_3$O-N=); X=COOH, Z=N; Z',Z", Z'" = CH (**methyloxime of 57**):

3-methyl-TTNCB methyl ester (151 mg, 0.41 mmol) in EtOH (1 mL) was treated with methoxylamine hydrochloride (52 mg, 0.62 mmol) and pyridine (70 μL, 0.82 mmol), and the mixture was heated

15   at reflux for 5 h. The reaction was worked-up in a manner identical to that described for **115** to give a solid (169 mg). The crude product was hydrolyzed in excess KOH/MeOH at ambient temperature for 24 h. The methanol was removed *in vacuo*. The residue was taken-up in water and the aqueous layer was adjusted to pH = 4-

20   5 with 1 M aqueous HCl. The aqueous solution was extracted 3 times with EtOAc; the organic layers were combined, and washed with water (2x) and brine. The organic solution was dried (NaSO$_4$), filtered, concentrated, and crystallized (Et$_2$O/hexanes) to give a white solid, 96 mg (61%). MP: 260-263°C; ^{1}H-NMR (CDCl$_3$) δ 1.22 (s, 6H,

25   2(CH$_3$)), 1.30 (s, 6H, 2(CH$_3$)), 1.68 (s, 4H, 2(CH$_2$)), 2.10 (s, 3H, CH$_3$), 4.08 (s, 3H, OCH$_3$), 6.99 (s, 1H, Ar-CH), 7.18 (s, 1H, Ar-CH), 7.63 (d, 1H, Py-CH, J = 8.0 Hz), 8.31 (d, 1H, Py-CH, J = 8.0 Hz), 9.31 (s, 1H, Py-CH).

Example 50

Preparation of compound **138**, where R1, R2, R3, R4, R5 are methyl;
R' and R" are n-butyloxime (n-BuO-N=); X = COOH (**n-butyloxime of 3-methyl-TTNCB**):

5   A solution of the oxime of 3-methyl-TTNCB (compound **112**,
121 mg, 0.33 mmol) in THF (0.3 mL) and DMPU (0.3 mL) was added at
0°C to a suspension of NaH (24 mg, 1.0 mmol) in THF (1.0 mL). The
suspension was allowed to warm to room temperature with stirring
over 30 minutes, then a solution of n-butyl bromide (136 mg, 110

10   μL, 1.0 mmol) in THF (1.0 mL) was added. The solution was allowed
to warm to room temperature and stirred for 15 hr. Aqueous,
saturated NH_4Cl (3.0 mL) was added and the aqueous layer was
adjusted to pH = 4-5 with 1 M aqueous HCl. The aqueous solution
was extracted 3 times with EtOAc; the organic layers were combined,

15   and washed with water (2x) and brine. The organic solution was
dried ($MgSO_4$), filtered, concentrated, and crystallized
(ether/hexanes) to give a white solid, 82 mg (58%). MP: 195-198°C;
^{1}H-NMR ($CDCl_3$) δ0.94 (t, 3H, CH_3, J = 7.4 Hz), 1.22 (s, 6H, 2(CH_3)),
1.31 (s, 6H, 2(CH_3)), 1.69 (s, 4H, 2(CH_2)), 1.72 (mult, 2H, CH_2, J =

20   7.3 Hz), 2.05 (s, 3H, CH_3), 4.16 (t, 3H, OCH_2, J = 6.7 Hz), 6.97 (s,
1H, Ar-CH), 7.16 (s, 1H, Ar-CH), 7.57 (d, 2H, Ar-CH_2, J = 8.4 Hz),
8.04 (d, 2H, Ar-CH_2, J = 8.4 Hz).

Example 51

Preparation of compound **139**, where R1, R2, R3, R4, R5 are methyl;
25   R' and R" are n-propyloxime (n-ProO-N=); X = COOH (**n-propyloxime of
3-methyl-TTNCB**):

          A solution of the oxime of 3-methyl-TTNCB (compound
number **112**, 121 mg, 0.33 mmol) in THF (0.3 mL) and DMPU (0.3 mL)
was added at 0°C to a suspension of NaH (24 mg, 1.0 mmol) in THF

30   (1.0 mL). The suspension was allowed to warm to room temperature

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with stirring over 30 minutes, then a solution of n-propyl bromide (122 mg, 90 μL, 1.0 mmol) was added. The solution was allowed to warm to room temperature and stirred for 15 hr. Aqueous, saturated NH_4Cl (5.0 mL) was added and the aqueous layer was adjusted to pH =

5 4-5 with 1 M aqueous HCl. The aqueous solution was extracted 3 times with EtOAc; the organic layers were combined, and washed with water (2x) and brine. The organic solution was dried ($MgSO_4$), filtered, concentrated, and crystallized (ether/hexanes) to give a white solid, 99 mg (73%). MP: 178-180°C; ^{1}H-NMR ($CDCl_3$) $\delta0.93$ (t,

10 3H, CH_3, J = 7.4 Hz), 1.26 (s, 6H, 2(CH_3)), 1.35 (s, 6H, 2(CH_3)), 1.51 (mult, 2H, CH_2, J = 7.4 Hz), 1.73 (mult, 6H, 2(CH_2)/CH_2), 2.09 (s, 3H, CH_3), 4.24 (t, 3H, OCH_2J = 7.4 Hz), 7.00 (s, 1H, Ar-CH), 7.19 (s, 1H, Ar-CH), 7.60 (d, 2H, Ar-CH_2, J = 8.5 Hz), 8.07 (d, 2H, Ar-CH_2, J = 8.5 Hz).

15 **Example 52**

Preparation of compound **140**, where R1, R2, R3, R4, R5 are methyl; R' and R" are cyanoimine (=N-CN); X = COOH (**cyanoimine of 3-methyl-TTNCB**);

A solution of 3-methyl-TTNCB (350 mg, 1.0 mmol) in

20 methylene chloride (1.5 mL) was treated with bis(trimethylsilyl)carbodiimide (186 mg, 230 μL, 1.0 mmol) at room temperature. The solution was cooled to 0°C, and treated with a 1M solution of $TiCl_4$ in methylene chloride (2 eq., 2 mL, 2 mmol). The resulting dark red solution was heated at reflux for 8 hr. An

25 additional equivalent of $TiCl_4$ (1 mL of 1M methylene chloride solution) was added and the reflux was continued for 3 hours, and TLC analysis indicated complete conversion to one product. The solution was cooled to room temperature, then poured into ice cold aqueous $NaHSO_4$. The mixture was diluted with ethyl acetate and

30 filtered through a pad of celite. The aqueous layer was washed

twice with ethyl acetate and the organic layers were combined. The organic solution was washed twice with water and once with saturated aqueous NaCl solution, dried ($Na_2 SO_4$), filtered, and concentrated. The residue was crystallized from solution with

5 methylene chloride/hexanes/benzene to give a pale yellow solid, 207 mg (55%). MP: 217-219°C; IR (KBr pellet) 3500-2600 br, 2961, 2924, 2666, 2210, 1693, 1582, 1560, 1427, 1408, 1314, cm^{-1}; ^{1}H-NMR (CDCl$_3$) δ 1.26 (s, 6H, 2(CH$_3$)), 1.32 (s, 6H, 2(CH$_3$)), 1.72 (s, 4H, 2(CH$_2$)), 2.11 (s, 3H, CH$_3$), 7.14 (s, 1H, Ar-CH), 7.24 (s, 1H, Ar-

10 CH), 7.90 (d, 2H, Ar-CH, J = 8.1 Hz), 8.17 (d, 2H, Ar-CH, J = 8.1 Hz); ^{13}C-NMR (CDCl$_3$) δ 191.3, 170.5, 148.6, 143.1, 140.4, 133.9, 132.7, 131.8, 130.4, 129.2, 125.9, 34.8, 34.7, 34.4, 34.1, 31.7, 31.6, 19.5; FAB MS $C_{24}H_{26}N_2O_2$: 375 (MH$^+$).

Example 53

15 Preparation of compound **141**, where R1, R2, R3, R4, R5 are methyl; R' and R" are allyloxime (allylO-N=); X = COOH (**allyloxime of 3-methyl-TTNCB**):

A solution of the oxime of 3-methyl-TTNCB (compound number **112**, 100 mg, 0.27 mmol) in THF (0.3 mL) and DMPU (0.3 mL)

20 was added at 0°C to a suspension of NaH (20 mg, 0.82 mmol) in THF (1.0 mL). The suspension was allowed to warm to room temperature with stirring over 30 minutes, then a solution of allyl bromide (71 μL, 0.82 mmol) was added. The solution was stirred at room temperature for an additional 12 hr. Aqueous, saturated NH$_4$Cl (5.0

25 mL) was added and the aqueous layer was adjusted to pH = 4-5 with 1 M aqueous HCl. The aqueous solution was extracted 3 times with EtOAc; the organic layers were combined, and washed with water (2x) and brine. The organic solution was dried (MgSO$_4$), filtered, concentrated, and crystallized (ether/hexanes) to give a white

30 solid, 87 mg (78%). ^{1}H-NMR (CDCl$_3$) δ 1.21 (s, 6H, 2(CH$_3$)), 1.31 (s,

6H, 2(CH$_3$)), 1.69 (s, 4H, 2(CH$_2$)), 2.06 (s, 3H, CH$_3$), 4.71 (d, 2H,

OCH$_2$, J = 5.6 Hz), 5.19 (dd, 1H, =CH$_{trans}$, J=10.4, 1.5 Hz), 5.27 (dd,

1H, = CH$_{cis,}$ J = 17.2, 1.5 Hz), 6.02 (mult, 1H, =CH), 6.97 (s, 1H,

Ar-CH), 7.16 (s, 1H, Ar-CH), 7.57 (d, 2H, Ar-CH, J = 8.2 Hz), 8.03

5   (d, 2H, Ar-CH, J = 8.2 Hz).

Example 54:

Preparation of compound **142**, where R1, R2, R3, R4, R5 are methyl;
R' and R" are 4-(3-methyl-butenyl-1-carboxy)oxime (HOOC-C=C(CH$_3$)CH$_2$-
O-N=); X = COOH **(4-(3-methyl-butenyl-1-carboxy)oxime of 3-methyl-**

10 **TTNCB)**:

A solution of the oxime of 3-methyl-TTNCB (compound
number **112**, 202 mg, 0.55 mmol) in THF (0.5 mL) and DMPU (0.5 mL)
was added at 0°C to a suspension of NaH (38 mg, 1.7 mmol) in THF
(1.7 mL). The suspension was allowed to warm to room temperature

15 with stirring over 30 minutes, then a solution of ethyl 4-bromo-3-
methyl-but-2-ene carboxylate (343 mg, 1.7 mmol) was added. The
solution was allowed to warm to room temperature and stirred for 12
hr. Aqueous, saturated NH$_4$Cl (5.0 mL) was added and the aqueous
layer was adjusted to pH = 4.5 with 1 M aqueous HCl. The aqueous

20 solution was extracted 3 times with EtOAc; the organic layers were
combined, and washed with water (2x) and brine. The organic
solution was dried (MgSO$_4$), filtered, and concentrated to give a
yellow oil. Radial chromatography (1 mm SiO$_2$ plate, 9 : 1 =
hexane; EtOAc with gradual addition of 2% isopropanol) gave a

25 yellow oil. The ester was hydrolyzed in excess KOH/MeOH at room
temperature for 24 hr. The methanol was removed *in vacuo*. The
residue was taken-up in water and the aqueous layer was adjusted to
pH ≈ 4-5 with 1 M aqueous HCl. The aqueous solution was extracted
3 times with EtOAc; the organic layers were combined, and washed

with water (2x) and brine. The organic solution was dried ($NaSO_4$), filtered, concentrated, and crystallized (Et_2O/hexanes) to give a white solid, 110 mg (43%). ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, 2(CH_3)), 1.31 (s, 6H, 2(CH_3)), 1.70 (s, 4H, 2(CH_2)), 2.06 (s, 3H, Ar-CH_3),

5   2.14 (s, 3H, CH_3), 4.71 (s, 2H, OCH_2), 5.85 (s, 1H, =CH), 7.01 (s, 1H, Ar-CH), 7.16 (s, 1H, Ar-CH), 7.55 (d, 2H, Ar-CH_2, J = 8.2 Hz), 8.02 (d, 2H, Ar-CH_2, J = 8.2 Hz).

Example 55

Preparation of compound **143**, where R1, R2, R3, R4, R5 are methyl;

10   R' and R" are 2-aminoethyloxime (Cl-NH_3+CH_2CH_2-O-N=); X = COOH **(2-aminoethyloxime of 3-methyl-TTNCB)**:

To a solution of the oxime of 3-methyl-TTNCB (compound number **112**, 108 mg, 0.30 mmol) and 2-bromoethylamine hydrobromide (182 mg, 0.89 mmol) in DMF (1 mL) was added excess powered KOH at

15   0°C. The yellow solution was allowed to warm to room temperature and stirred for 48 hr. Aqueous, saturated NH_4Cl (5.0 mL) was added and the aqueous layer was adjusted to pH = 2 with 1 M aqueous HCl. The aqueous solution was extracted 3 times with EtOAc; the organic layers were combined, and washed with water (2x) and brine. The

20   organic solution was dried ($MgSO_4$), filtered, and concentrated, and recrystallized (Et_2O/hexanes) to give a white solid, 64 mg (50%). IR (KBr pellet) 3600-3200 broad, 3420, 2922, 2855, 1695, 1591, 1456, 1364, 1231, 1017 cm^{-1}; ^{1}H-NMR ($CDCl_3$/CD_3OD) δ 1.21 (s, 6H, 2(CH_3)), 1.29 (s, 6H, 2(CH_3)), 1.70 (s, 4H, 2 (CH_2)), 2.06 (s, 3H,

25   Ar-CH_3), 2.14 (s, 3H, CH_3), 3.29 (m, 2H, CH_2), 4.37 (m, 2H, CH_2), 7.00 (s, 1H, Ar-CH), 7.24 (s, 1H, Ar-CH), 7.50 (d, 2H, Ar-CH_2, J = 8.0 Hz), 7.94 (d, 2H, Ar-CH_2, J = 8.0 Hz).

Evaluation of Retinoid Receptor Subtype Selectivity

Representative synthetic retinoid compounds of the current invention were analyzed and found to exhibit subtype selectivity for retinoid receptors, and to be capable of modulating

5   processes selectively mediated by retinoid X receptors, as discussed more fully below.

As employed herein, the phrase "processes selectively mediated by retinoid X receptors" refers to biological, physiological, endocrinological, and other bodily processes which

10   are mediated by receptors or receptor combinations which are responsive to retinoid X receptor selective processes, *e.g.*, compounds which selectively activate one and/or multiple members of the RXR subfamily. Modulation includes activation or enhancement of such processes as well as inhibition or repression, and can be

15   accomplished *in vitro* or *in vivo*. *In vivo* modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like. It is well accepted that modulation of such processes has direct relevance to use in treating disease states.

20   The receptors which are responsive to retinoid X receptor selective ligands include: retinoid X receptor-alpha, retinoid X receptor-beta, retinoid X receptor-gamma, and splicing variants encoded by the genes for such receptors, as well as various combinations thereof (*i.e.*, homodimers, homotrimers, heterodimers,

25   heterotrimers, and the like). Also included are combinations of retinoid X receptors with other members of the steroid/thyroid superfamily of receptors with which the retinoid X receptors may interact by forming heterodimers, heterotrimers, and the higher heteromultimers. For example, the retinoic acid receptor-alpha,

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-beta, or -gamma isoforms form a heterodimer with any of the
retinoid X receptor isoforms, (*i.e.*, alpha, beta, or gamma, including
any combination of the different receptor isoforms), and the
various retinoid X receptors form a heterodimer with thyroid

5   receptor and form a heterodimer with vitamin D receptor. Members
of the retinoid X receptor subfamily form a heterodimer with
certain "orphan receptors" including PPAR (Issemann and Green,
Nature, 347:645-49 (1990)); HNF4 (Sladek *et al., Genes & Development* 4:2353-65
(1990)); the COUP family of receptors (*e.g.*, Miyajima *et al.*, *Nucleic Acids*

10   *Research* 16:11057-74 (1988), and Wang *et al., Nature,* 340:163-66 (1989));
COUP-like receptors and COUP homologs, such as those described by
Mlodzik *et al.* (*Cell*, 60:211-24 (1990)) and Ladias *et al. (Science,* 251:561-65
(1991)); the ultraspiracle receptor (*e.g.,* Oro *et al., Nature,* 347:298-301
(1990)); and the like.

15   As employed herein, the phrase "members of the
steroid/thyroid superfamily of receptors" (also known as "nuclear
receptors" or "intracellular receptors") refers to hormone binding
proteins that operate as ligand-dependent transcription factors.
Furthermore, this classification includes identified members of the

20   steroid/thyroid superfamily of receptors for which specific ligands
have not yet been identified (referred to hereinafter as "orphan
receptors"). All members of the intracellular receptor superfamily
have the intrinsic ability to bind to specific DNA sequences.
Following binding, the transcriptional activity of a target gene

25   (*i.e.*, a gene associated with the specific DNA sequence) is modulated
as a function of the ligand bound to the receptor. Also, see
Heyman *et al., Cell,* 68:397-406 (1992), and copending U.S. Serial No.
809,980, filed December 18, 1991, whose entire disclosures are
incorporated herein by reference.

The modulation of gene expression by the ligand retinoic acid and its receptors can be examined in a reconstituted system in cell culture. Such a system was used to evaluate the synthetic retinoid compounds of this invention for their interaction with the

5   retinoid receptor subtypes RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ.

The system for reconstituting ligand-dependent transcriptional control, which was developed by Evans *et al., Science,* 240:889-95 (1988), has been termed a "co-transfection" or *"cis-trans"* assay. This assay is described in further detail in U.S. Patent

10   Nos. 4,981,784 and 5,071,773, which are incorporated herein by reference. Also see Heyman *et al., Cell,* 68:397-406 (1992). The co-transfection assay provides a mechanism to evaluate the ability of a compound to modulate the transcription response initiated by an intracellular receptor. The co-transfection assay is a functional,

15   rapid assay that monitors hormone or ligand activity, is a good predictor of an *in vivo* system, and can be used to quantitate the pharmacological potency and utility of such ligands in treating disease states. Berger *et al., J. Steroid Biochem. Molec. Biol.,* 41:733-38 (1992).

20   Briefly, the co-transfection assay involves the introduction of two plasmids by transient transfection into a retinoid receptor-negative mammalian cell background. The first plasmid contains a retinoid receptor cDNA and directs constitutive expression of the encoded receptor. The second plasmid contains a

25   cDNA that encodes for a readily quantifiable protein, *e.g.,* firefly luciferase or chloramphenicol acetyl transferase (CAT), under control of a promoter containing a retinoid acid response element, which confers retinoid dependence on the transcription of the reporter. In this co-transfection assay, all retinoid receptors

30   respond to all-*trans*-retinoic acid in a similar fashion. This assay

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can be used to accurately measure efficacy and potency of retinoic acid and synthetic retinoids as ligands that interact with the individual retinoid receptor subtypes.

5   Accordingly, synthetic retinoid compounds of the current invention were evaluated for their interaction with retinoid receptor subtypes using the co-transfection assay in which CV-1 cells were co-transfected with one of the retinoid receptor subtypes, a reporter construct, and an internal control to allow normalization of the response for transfection efficiency. The
10   following example is illustrative.

Example 56

          Retinoids: All-*trans*-retinoic acid (RA) and 13-*cis*-retinoic acid (13-*cis*-RA) were obtained from Sigma. 9-*cis*-retinoic acid (9-*cis*-RA) was synthesized as described in Heyman *et al.*, *Cell*, 68:397-406 (1992).
15   Retinoid purity was established as greater than 99% by reverse phase high-performance liquid chromatography. Retinoids were dissolved in dimethylsulfoxide for use in the transcriptional activation assays.

          Plasmids: The receptor expression vectors used in the co-
20   transfection assay have been described previously (pRShRAR-α: Giguere *et al.* (1987); pRShRAR-β and pRShRAR-γ: Ishikawa *et al.* (1990); pRShRXR-α: Mangelsdorf *et al.*, (1990); pRSmRXR-β and pRSmRXR-γ: Mangelsdorf *et al., Genes & Devel.*, 6:329-44 (1992)). A basal reporter plasmid Δ-MTV-LUC (Hollenberg and Evans, *Cell*, 55:899-906 (1988))
25   containing two copies of the TRE-palindromic response element 5'-TCAGGTCATGACCTGA-3' (Umesono *et al., Nature,* 336:262-65 (1988)) was used in transfections for the RARs, and CRBPIIFKLUC, which contains an RXRE (retinoid X receptor response element (Mangelsdorf *et al.*, *Cell*, 66:555-61 (1991)), was used in transfections for the RXRs.

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Co-transfection Assay In CV-1 Cells: A monkey kidney cell
line, CV-1, was used in the *cis-trans* assay. Cells were transfected
with two plasmids. The trans-vector allowed efficient production
of the retinoid receptor in these cells, which do not normally

5   express this receptor protein. The *cis*-vector contains an easily
assayable gene product, in this case the firefly luciferase,
coupled to a retinoid-responsive promoter, *i.e.*, an RARE or RXRE.
Addition of retinoic acid or an appropriate synthetic retinoid
results in the formation of a retinoid-RAR or -RXR complex that

10   activates the expression of luciferase gene, causing light to be
emitted from cell extracts. The level of luciferase activity is
directly proportional to the effectiveness of the retinoid-receptor
complex in activating gene expression. This sensitive and
reproducible co-transfection approach permits the identification of

15   retinoids that interact with the different receptor isoforms.

Cells were cultured in DMEM supplemented with 10%
charcoal resin-stripped fetal bovine serum, and experiments were
conducted in 96-well plates. The plasmids were transiently
transfected by the calcium phosphate method (Umesono and Evans, *Cell,*

20   57:1139-46 (1989) and Berger *et al., J. Steroid Biochem. Molec. Biol.*, 41:733-38
(1992)) by using 10 ng of a pRS (Rous sarcoma virus promoter)
receptor-expression plasmid vector, 50 ng of the reporter
luciferase (LUC) plasmid, 50 ng of pRSβ-GAL(β-galactosidase) as an
internal control, and 90 ng of carrier plasmid, pGEM. Cells were

25   transfected for 6 h and then washed to remove the precipitate. The
cells were then incubated for 36 h with or without retinoid. After
the transfection, all subsequent steps were performed on a Beckman
Biomek Automated Workstation. Cell extracts were prepared, then
assayed for luciferase and β-galactosidase activities, as described

30   by Berger *et al.* (1992). All determinations were performed in

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triplicate in two independent experiments and were normalized for transfection efficiency by using β-galactosidase as the internal control. Retinoid activity was normalized relative to that of all-*trans*-retinoic acid and is expressed as potency (EC50), which is the

5   concentration of retinoid required to produce 50% of the maximal observed response, and efficacy (%), which is the maximal response observed relative to that of all-*trans*-retinoic acid at 10^{-5}M. The data obtained is the average of at least four independent experiments. Efficacy values less than 5% are not statistically

10   different than the 0% background. Compounds with an efficacy of less than 20% at concentrations of 10^{-5} M are considered to be inactive. At higher concentrations of compound, such as 10^{-4} M, these compounds are generally toxic to cells and thus the maximal efficacy at 10^{-5} M is reported in the tables and figures contained

15   herein.

The synthetic retinoid compound 3-methyl-TTNCB, as described above, was evaluated for its ability to regulate gene expression mediated by retinoid receptors. As shown in Figure 1, this compound is capable of activating members of the RXR

20   subfamily, *i.e.*, RXRα, RXRβ, and RXRγ, but clearly has no significant activity for members of the RAR subfamily, *i.e.*, RARα, RARβ, and RARγ. Assays using all-*trans*-retinoic acid (Figure 2) and 9-*cis*-retinoic acid (Figure 3) were run for reference, and demonstrate that these retinoic acid isomers activate members of both the RAR

25   and RXR subfamilies.

Potency and efficacy were calculated for the 3-methyl-TTNCB compound, as summarized in the following table. For reference, the data for 9-*cis*-retinoic acid are also included.

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TABLE 1

	Potency (nM)	Efficacy
3-Methyl-TTNCB		
RXRα	330	130%
RXRβ	200	52%
RXRγ	260	82%
RARα	>10,000	<2%
RARβ	>10,000	<4%
RARγ	>10,000	<4%
9-cis-retinoic acid		
RXRα	150	140%
RXRβ	100	140%
RXRγ	110	140%
RARα	160	100%
RARβ	5	82%
RARγ	47	120%

As shown by the data in Table 1, 3-methyl-TTNCB readily and at low concentrations activates RXRs. Further, 3-methyl-TTNCB is more potent an activator of RXRs than RARs, and preferentially activates RXRs in comparison to RARs, in that much higher concentrations of the compound are required to activate the RARs. In contrast, 9-cis-retinoic acid does not preferentially activate the RXRs, as also shown in Table 1. Rather, 9-cis-retinoic acid activates the RARβ and RARγ isoforms at lower concentrations and more readily than the RXRβ and RXRγ isoforms, and has substantially the same, within the accuracy of the measurement, activity for the RARα isoform in comparison to the RXRα isoform.

An extract reported to contain 9-cis-retinoic acid has previously been reported as at least 10-fold more potent in inducing RXRα than RARα (Heyman et al., Cell, 68:397,399 (January 24, 1992)). Presently available data indicate that 9-cis-retinoic acid does not preferentially activate RXRs in comparison to RARs, as

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shown and discussed above. The compounds of this invention
preferentially activate RXRs in comparison to RARs, and are
preferably at least three times more potent as activators of RXRs
than RARs, and more preferably at least five times more potent as
5   activators of RXRs than RARs.

Potency and efficacy have also been calculated for the 3-
methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP,
TPNEP, and TPNCP compounds, as summarized below in Table 2.

TABLE 2

		Potency (nM)	Efficacy
10			
	3-Methyl-TTNEB		
	RXRα	40	83%
	RXRβ	21	102%
	RXRγ	34	80%
15	RARα	>10,000	6%
	RARβ	>10,000	17%
	RARγ	>10,000	19%
	3-Bromo-TTNEB		
	RXRα	64	88%
20	RXRβ	54	49%
	RXRγ	52	71%
	RARα	>10,000	3%
	RARβ	>10,000	18%
	RARγ	>10,000	15%
25	**3-Methyl-TTNCHBP**		
	RXRα	1100	113%
	RXRβ	1100	155%
	RXRγ	300	128%
	RARα	>10,000	<2%
30	RARβ	>10,000	7%
	RARγ	>10,000	17%

3-Methyl-TTNEHBP (63)

RXRα	140	125%
RXRβ	71	121%
RXRγ	48	163%
RARα	>10,000	<2%
RARβ	1,900	25%
RARγ	>10,000	10%

5

TPNEP (58)

RXRα	5	75%
RXRβ	5	138%
RXRγ	6	100%
RARα	>10,000	<2%
RARβ	>10,000	<2%
RARγ	1,500	24%

10

15 **TPNCP (62)**

RXRα	4	63%
RXRβ	4	93%
RXRγ	3	49%
RARα	>10,000	<2%
RARβ	>10,000	<2%
RARγ	>10,000	<2%

20

As shown by the data in Table 2, 3-methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP, TPNEP, and TPNCP each readily and preferentially activate the RXRs, and are more potent

25   as activators of RXRs than of RARs. The diminished activity of these compounds for the RARs in comparison to the RXRs is also shown for some of these compounds in Figures 4-7.

The potency and efficacy of the oxime derivative compounds **112** and **115** have also been calculated, as summarized

30   below in Table 3.

TABLE 3

	Potency (nM)	Efficacy
Oxime Cd. 112		
RXRα	15	66%
RXRβ	8	51%
RXRγ	12	62%
RARα	>10,000	3%
RARβ	>10,000	3%
RARγ	>10,000	3%
Oxime Cd. 115		
RXRα	5	61%
RXRβ	5	71%
RXRγ	5	70%
RARα	>10,000	4%
RARβ	>10,000	2%
RARγ	>10,000	3%

As shown, oxime compounds **112** and **115** preferentially activate RXRs in comparison to RARs.

The selective activity of the compounds of this invention for Retinoid X Receptors is not exhibited by other known compounds. For example, compounds such as those described in U.S. Patent No. 4,833,240 (Maignan et al.) appear structurally similar to the compounds of this invention, but lack a functional group (such as methyl, ethyl, isopropyl, bromo, chloro, etc.) at the 3-position. Such compounds have little or no potency and lack any selectivity for RXRs.

For example, a representative compound of U.S. Patent No. 4,833,240 (Maignan) is shown below, along with the compound 3-methyl-TTNCB of this invention.
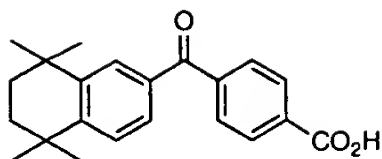
Maignon Ex. II
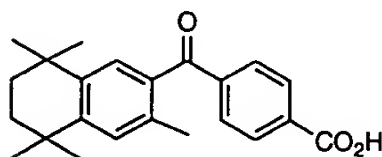
3-methyl-TTNCB

The potency and efficacy of the Maignon compound and that of 3-methyl-TTNCB are summarized below:

	Potency (nM)	Efficacy
Maignon Ex. II		
RXRα	3,000	82%
RXRβ	3,000	44%
RXRγ	3,000	64%
RARα	>10,000	11%
RARβ	1,900	58%
RARγ	2,000	56%
3-Methyl-TTNCB		
RXRα	330	130%
RXRβ	200	52%
RXRγ	260	82%
RARα	>10,000	<2%
RARβ	>10,000	<4%
RARγ	>10,000	<4%

As shown, the Maignon compound is virtually inactive and shows no selectivity for RXRs. In contrast, the compounds of this

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invention such as 3-methyl-TTNCB, which have a substituant at the 3-position, are potent activators of RXRs and exhibit the unexpected RXR selectivity shown in Table 1 (as well as Tables 2 and 3) and discussed above.

5   It can be expected that synthetic retinoid ligands, such as those exemplified in Tables 1, 2, and 3 which preferentially affect some but not all of the retinoic acid receptor isoforms, can, in pharmacological preparations, provide pharmaceuticals with higher therapeutic indices and a better side effect profile than

10   currently used retinoids. For example, the compounds of the present invention have been observed to be less irritating to the skin than previously known 95
retinoids.

          The retinoid compounds of this invention are useful for

15   the treatment of certain dermatological conditions such as keratinization disorders, *i.e.*, differentiation/
proliferation. A standard assay to determine the activity of these compounds is the measurement of the enzymatic activity for transglutaminase; this is a measure of the antiproliferative action

20   of retinoids. Retinoids have been shown to inhibit the pathway of differentiation, which is indicated by a decrease in several biochemical markers that are associated with the expression of squamous cell phenotype, such as transglutaminase. (Yuspa *et al.*, *Cancer Research*, 43:5707-12 (1983)). As can be seen from Figure 8, the

25   3-methyl-TTNCB compound is capable of inhibiting transglutaminase activity and inhibits 50% of the enzyme activity at 1×10^{-7} M.

          The retinoid compounds of this invention have been demonstrated in *in vitro* tests to block (or antagonize) AP-1 activity, a set of oncogenes which drive cellular proliferation. Many

30   proliferative disorders are the results of oncogenes/oncogene

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activation, and therefore a compound which blocks the AP-1 oncogene pathway can be used to treat diseases associated with proliferative disorders including cancers, inflammatory diseases, psoriasis, etc. For example, the compound 3-methyl-TTNEB was evaluated using the

5   co-transfection assay in which HeLa cells were co-transfected with a plasmid expressing RXRα under the control of a constitutive promoter, and a plasmid which expresses the reporter enzyme luciferase under the control of a conditional promoter (collagenase) containing an AP-1 responsive element. *E.g.*, Angel *et*

10 *al.*, *Mol. Cell. Biol.*, 7:2256 (1987); Lafyatis *et al.*, *Mol. Endocrinol.*, 4:973 (1990). Following AP-1 activation, the assay results showed antagonism of AP-1 activity by the 3-methyl-TTNEB compound via RXRα in a dose-dependent manner. Other compounds of this invention have shown similar antagonism of AP-1 activity. These results

15 demonstrate that RXR-selective compounds such as 3-methyl-TTNEB can be used as anti-proliferatives to limit cell growth and treat diseases associated with hyperproliferation.

The compounds of this invention also exhibit good comedolytic activity in the test on Rhino mice described by Kligman

20 *et al. (J. of Inves. Derm.*, 73:354-58 (1979)) and Mezick *et al. (J. of Inves. Derm.*, 83:110-13 (1984)). The test on Rhino mice has been a model for screening comedolytic agents. The activity of the 3-methyl-TTNCB retinoid compound, as well as 9-*cis* and all-*trans* retinoic acid is shown in Figure 9. A 0.1% solution of 3-methyl-TTNCB is capable of

25 inhibiting the utriculi diameter by approximately 50%. It has also been observed that 3-methyl-TTNCB is less irritating to the skin of Rhino mice than 9-*cis*- or all-*trans*-retinoic acid.

The co-transfection assay allows examination of the ability of a compound to modulate gene expression in a retinoid

30 receptor dependent fashion. To examine the ability of the

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compounds of this invention to directly interact with the
receptors, we have examined the ligand binding properties of all
six retinoid receptors. The receptors were expressed employing a
baculovirus expression system in which we have demonstrated that

5   RXRα binds 9-*cis*-retinoic acid with high affinity (Heyman *et al., Cell,*
68:397 (1992)). The binding parameters of receptors expressed in
baculovirus systems and mammaliam systems are essentially
identical.

The synthetic retinoids of the current invention have

10 also been tested using radioligand displacement assays. By testing
the abilities of various synthetic retinoids to compete with the
radiolabeled retinoic acid for binding to various receptor
isoforms, the ability of the compounds to directly interact with
the receptor can be examined, and the relative dissociation

15 constant for the receptor itself can be determined. This is an
important supplementary analysis to the co-transfection assay since
it can detect different properties/determinants of retinoid
activity than are measured in the co-transfection assay. These
determinants/differences in the two assay systems may include (1)

20 activating or inactivating metabolic alterations of the test
compounds, (2) binding to serum proteins which could alter the free
concentration or other properties of the test compound, (3)
differences in cell permeation among test compounds, (4) intrinsic
differences in the affinity of the test compounds for the receptor

25 proteins, *i.e.*, in K_d, can be directly measured, and (5)
conformational changes produced in the receptor after binding of
the test compound, reflected in the effects on reporter gene
expression; (*i.e.*, a functional measurement of receptor activation).

The 3-methyl-TTNCB compound is capable of displacing ^{3}H-

30 9-*cis*-retinoic acid bound to the RXRs, but is not capable of

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displacing radiolabeled ligand that is bound to the RARs. This

indicates that the 3-methyl-TTNCB compound preferentially binds

RXRs in comparison to RARs, a property which would be expected of a

ligand selective for the RXRs.

5   The K_d values were determined by application of the

Cleng-Prusoff equation. These values were based on a determination

of the IC_{50} value as determined graphically from a log-logit plot of

the data.

          Binding data were obtained for various compounds using

10   the method discussed in Wecksler & Norman, *Anal. Biochem.* 92:314-23

      (1979). Results are shown below in Table 4.

TABLE 4

	3-Methyl-TTNCB	Binding (Kd_{50} nM)
	RXRα	350
15	RXRβ	230
	RXRγ	365
	RARα	>10,000
	RARβ	>10,000
	RARγ	>10,000
20	3-Methyl-TTNEB	
	RXRα	41
	RXRβ	20
	RXRγ	22
	RARα	5,500
25	RARβ	5,400
	RARγ	3,200
	TPNEP (58)	
	RXRα	22
	RXRβ	21
30	RXRγ	39
	RARα	7,800
	RARβ	4,900
	RARγ	6,000

TPNCP (62)

RXRα	3
RXRβ	3
RXRγ	3
RARα	>10,000
RARβ	>10,000
RARγ	>10,000

Oxime Cd. 112

RXRα	6
RXRβ	5
RXRγ	5
RARα	8,700
RARβ	>10,000
RARγ	>10,000

The compounds in Table 4 were shown to readily and preferentially activate RXRs, and to be more potent as activators of RXRs than of RARs using the co-transfection assay, as discussed above. The binding results in Table 4 show these compounds to also preferentially bind RXRs versus RARs. Taken together the ligand binding properties of these compounds and their ability to selectively modulate members of the RXR subfamily demonstrate the identification of a class of compounds with the unique biological properties. The binding properties and especially the transcriptional activation assays are a good predictor of the pharmacological activity of a compound (Berger *et al.* (1992)).

It has been recognized that the co-transfection assay provides a functional assessment of the ligand being tested as either an agonist or antagonist of the specific genetic process sought to be affected, and is a predictor of *in vivo* pharmacology (Berger et al. (1992)). Ligands which do not significantly react with other intracellular receptors, as determined by the co-transfection assay, can be expected to result in fewer pharmacological side effects. Because the co-transfection assay is run in living cells, the evaluation of a ligand provides an early

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indicator of the potential toxicity of the candidate at concentrations where a therapeutic benefit would be expected.

Processes capable of being modulated by retinoid receptors, in accordance with the present invention, include *in vitro*

5   cellular differentiation, the regulation of morphogenetic processes including limb morphogenesis, regulation of cellular retinol binding protein (CRBP), and the like. As readily recognized by those of skill in the art, the availability of ligands for the retinoid X receptor makes it possible, for the first time, to

10   elucidate the processes controlled by members of the retinoid X receptor subfamily. In addition, it allows development of assays for the identification of antagonists for these receptors.

The processes capable of being modulated by retinoid receptors, in accordance with the present invention, further

15   include the *in vivo* modulation of lipid metabolism; *in vivo* modulation of skin related processes (*e.g.*, acne, psoriasis, aging, wrinkling, and the like); *in vivo* modulation of programmed cell death (apoptosis); *in vivo* modulation of malignant cell development, such as occurs, for example, in acute promyelocytic leukemia, mammary cancer, prostate

20   cancer, lung cancer, cancers of the aerodigestive pathway, skin cancer, bladder cancer, and sarcomas; *in vivo* modulation of premalignant lesions, such as occurs with oral leukoplakia and the like; *in vivo* modulation of auto-immune diseases such as rheumatoic arthritis; *in vivo* modulation of fatty acid metabolism; and the like.

25   Such applications can be expected to allow the modulation of various biological processes with reduced occurrence of undesirable side effects such as teratogenic effects, skin irritation, mucosal dryness, lipid disturbances, and the like. *In vivo* applications can be employed with a wide range of subjects, such as, for example,

30   humans, rodents, sheep, pigs, cows, and the like.

For example, regarding the *in vivo* modulation of lipid metabolism referred to above, apolipoprotein A-1 ("apoA1") is a major protein component of plasma high density lipoprotein (HDL) cholesterol. The circulating level of HDL in humans has been shown

5   to be inversely correlated to the risk of atherosclerotic cardiovascular disease (ASCVD), the leading cause of morbidity and mortality in the United States, with a 3-4% increase in ASCVD for every 1% decrease in HDL cholesterol. Gordon *et al., New Engl. J. Med.*, 321:1311 (1989). While there are currently no good therapeutic

10 regimens that increase HDL cholesterol, it can be expected that regulating synthesis of apoA1 can be utilized to affect plasma concentrations of HDL cholesterol and to decrease the risk of ASCVD. Rubin *et al., Nature*, 353:265 (1991).

It has been established that regulation of transcription

15 of apoA1 is controlled by members of the intracellular receptor superfamily, and further that the apoA1 gene transcription start site "A" is a highly selective retinoic acid-responsive element that responds to retinoid X receptors. Rottman *et al., Mol. Cell. Biol.*, 11:3814-20 (1991). Because RXRs can form heterodimers with

20 transrepressers such as ARP-1 and COUP-TF and transactivators such as HNF-4, and an RXR response element resides in the apoA1 promoter, retinoids or ligands which selectively activate members of the RXR family of retinoic acid receptors may regulate apoA1 transcription. We have demonstrated in *in vivo* studies that ligands

25 of this invention which have selective activity for RXRs can be used to modulate apoA1/HDL cholesterol and to significantly raise plasma HDL levels, as demonstrated in the following example.

Example 57

Male Sprague-Dawley rats (160-200 gram) were obtained from Harlan. Animals were fed standard laboratory diets (Harlan/Teklad) and kept in an environmentally controlled animal

5   house with a light period lasting from 6 a.m. to 6 p.m. Animals were treated with drugs prepared as suspensions in olive oil.

To verify that RXR activation can increase plasma apoA1/HDL cholesterol, an initial study was carried out that included dosing rats for 4 days with an RAR-selective compound,

10   all-*trans* retinoic acid, the non-selective RAR/RXR agonist, 9-*cis*-retinoic acid, and either of two RXR-selective agents, 3-methyl-TTNCB or 3-methyl-TTNEB. Each drug was administered at a dose of 100 mg/kg, i.p. Positive control groups received olive oil as a vehicle. Twenty-four hours after the last treatment, rats were

15   sacrificed by CO_2 inhalation, blood was collected from the inferior vena cava into a tube containing 0.1 ml of 0.15% EDTA and centrifuged at 1500 x g for 20 min. at 4°C. Plasma was separated and stored at 4°C for evaluation of plasma total cholesterol and high density lipoprotein cholesterol (HDL-cholesterol).

20   Plasma total cholesterol was measured enzymatically utilizing Boeringer Mannheim Diagnostics High Performance Cholesterol Methods with an ABBOTT VP Bichromatic Analyzer. HDL cholesterol was measured after preparation of the HDL-containing fraction by heparin-manganese precipitation of plasma. HDL-

25   cholesterol in this fraction was estimated as mentioned earlier. All HDL separations were checked for contamination by other lipoproteins with agarose gel electrophoresis.

The results of this study are shown in Figure 10. As shown, rats receiving the RXR-selective compounds exhibited

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substantial and statistically significant increases in HDL levels, particularly when receiving 3-methyl-TTNEB.

Because the RXR-selective ligand 3-methyl-TTNEB was the most efficacious, additional 4 day experiments were conducted with

5   this agent at doses of 0.3, 1, 3, 6, 10, 30, 100, or 300 mg/kg i.p. in 1.0 ml olive oil or 1, 3, 10, 30, 100, 300 mg/kg p.o. in 1.0 ml olive oil for 4 days. An additional 30 day p.o. study was conducted with 10, 30, or 100 mg/kg 3-methyl-TTNEB to determine whether tolerance would develop to its pharmacological actions. For

10 the rats receiving 3-methyl-TTNEB in various doses for four days, it was observed that 3-methyl-TTNEB increased plasma concentrations of HDL-cholesterol in a dose-dependent manner with significant increases being made at the lowest dose, 0.3 mg/kg i.p. At its optimally efficacious dose, 3-methyl-TTNEB increased plasma HDL-

15 cholesterol concentrations from 58 mg/dl to 95 mg/dl - an increase of greater than 60%. Measurement of total cholesterol showed an increase due to the increase of the HDL-cholesterol fraction. Measurement of triglycerides showed either no change or a slight decrease. The 30-day study with 3-methyl-TTNEB did not indicate

20 development of tolerance to its pharmacological action.

This study demonstrated that 3-methyl-TTNEB increased plasma concentrations of HDL-cholesterol in a dose-dependent manner following either i.p. or p.o. administration.

The effect on circulating apoAl of orally administered 3-

25 methyl-TTNEB was also studied. Male Sprague-Dawley rats were treated daily with 3 mg/kg body weight of 3-methyl-TTNEB for four days. Serum samples were taken and analyzed by Western Blot using antisera specific to rat apoAl. The treatment with 3-methyl-TTNEB resulted in a significant increase in circulating apoAl level.

30 These studies demonstrate that treatment with an RXR specific compound such as 3-methyl-TTNEB increases plasma

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concentrations of apoA1/HDL cholesterol. Since such animal studies are an accepted predictor of human response, it would therefore be expected that such compounds could be used to therapeutically increase HDL-cholesterol in patients who either have, or are at

5   risk for, atherosclerosis.

Additional *in vitro* studies were also performed utilizing the co-transfection assay previously described within this application to demonstrate the effect of RXR-selective ligands on regulation of transcription of apoA1, as described in the following example.

10   **Example 58**:

This work focused on studying the transcriptional properties of the retinoid receptors RAR and RXR on a reporter molecule (*e.g.*, luciferase) under control of a basal promoter containing the RXR response element from the apoA1 gene ("A" site).

15   Plasmid constructs coding for the various receptors were transfected into a human hepatocyte cell line (HepG-2) along with the reporter plasmid. Reporter plasmids contained multimers of the apoA1 "A" site (-214 to -192 relative to transcription start site) shown to bind RXR. Widom *et al., Mol. Cell. Biol.* 12:3380-89 (1992); Ladias

20   & Karathanasis, *Science* 251:561-65 (1991). After transfection, treatment, harvest, and assay, the data obtained was normalized to transfected beta-galactosidase activity so as to control for transfection efficiency. The results demonstrated activation in the system with the RXR-specific ligands 3-methyl-TTNCB and 3-

25   methyl-TTNEb in a concentration-dependent fashion, demonstrating that the RXR specific ligands could regulate the transcriptional properties via the "A" site from the apoA1 gene. These compounds had no effect when RAR was used in the transfection, demonstrating

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receptor specificity. The transcriptional regulation by RXR was dependent on the presence of the hormone response element.

These *in vivo* and *in vitro* studies demonstrate that the RXR-selective compounds of this invention can be used to elevate

5   apoA1/HDL cholesterol and in the therapeutic treatment of related cardiovascular disorders.

Regarding the modulation of programmed cell death (apoptosis), the retinoid compounds of this invention have been demonstrated to induce apoptosis in particular cell types including

10   leukemic cells and squamous epithelial carcinomas. Normally in cells there is a fine balance between cellular processes of proliferation, differentiation, and cell death, and compounds which affect this balance may be used to treat certain cancers. Specifically, the ability of 3-methyl-TTNEB to induce

15   differentiation, inhibit proliferation, and induce apoptosis in an acute promyelucytic leukemia cell line, HL60, was studied. Cellular proliferation was measured by a thymidine incorporation assay (Shrivastav *et al.*, *Cancer Res.*, 40:4438 (1980)), and 3-methyl-TTNEB was found to have no effect on cellular proliferation. This

20   contrasts all-*trans*-retinoic acid, which inhibits thymidine incorporation. Cellular differation was measured by the ability of the cells to reduce nitroblue tetrazolium (NBT) (Breitman *et al.*, *Proc. Natl. Acad. Sci.*, 77:2936 (1980)), and 3-methyl-TTNEB was found not to induce undue differentiation. The EC_{50} for 3-methyl-TTNEB mediated

25   differentiation was >1000 μM, compared to 2.0μM for all-*trans*-retinoic acid. However, 3-methyl-TTNEB was found to induce transglutaminase activity in the HL60 cells (Murtaugh *et al.*, *J. Biol. Chem.* 258:11074 (1983)) in a' concentration-dependent manner, which correlates with the induction of apoptosis or programmed cell

30   death. It was further found that 3-methyl-TTNEB was able to induce

apoptosis as measured by DNA fragmentation and morphological changes. Other retinoid compounds of this invention showed similar results, and similar results were also shown in other cell lines such as squamous epithelial cell lines and ME180 cells, a human

5   cervical carcinoma.

These results show that RXR specific compounds such as 3-methyl-TTNEB induce apoptosis with a minimal direct effect on inhibition of proliferation and differentiation induction. Compounds which are capable of inducing apoptosis have been shown

10   to be effective in cancer chemotherapy (*e.g.*, anti-hormonal therapy for breast and prostate cancer).

In contrast, in another cell type, retinoids have been shown to inhibit activation driven T-cell apoptosis and 9-*cis*-retinoic acid was approximately ten fold more potent than

15   all-*trans*-retinoic acids (Ashwell *et al., Proceedings National Academy of Science, Vol. 90, p. 6170-6174* (1993)). These data imply that RXRs are involved in this event. Thus retinoids could be used to block and/or immunomodulate T-cell apoptosis associated with certain disease states (*e.g.*, AIDS).

20   It has been surprisingly found that the administration of a ligand which has specific activity for RXRs but essentially no activity for RARs, in combination with a ligand that has specific activity for RARS but not RXRs, provides a cellular response at extremely low dosages, dosages at which the ligands individually

25   provide no significant response. Specifically, the concentration-related effect of an RXR-specific ligand and a RAR-specific ligand on proliferation of a myeloma cell line (RPMI 8226) was studied in *in vitro* studies using a thymidine incorporation assay. This assay examines the incorporation of radiolabeled thymidine into DNA, and

30   by determining the ability of a compound to inhibit thymidine

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incorporation into DNA, provides a measure of cell proliferation.
(L.M. Bradley, Selected Methods in Cellular Immunology, Ch. 10.1,
pp. 235-38, Mishell & Shiigi (eds.), Freeman & Co., New York,
1980). Compounds which inhibit cell proliferation have well-known
5   utility in the treatment of certain cancers.

As shown previously (Table 2), 3-methyl-TTNEB activates
members of the RXR subfamily and has no significant activity for
members of the RAR subfamily. Examination of the effects of 3-
methyl-TTNEB on the proliferation of myeloma cells show a
10   concentration dependent inhibition of thymidine incorporation. The
IC_{50} (the concentration of 3-methyl-TTNEB required to produce 50%
inhibition of the maximal response) is 10^{-7} M, as shown in Figure
11. Concentrations less than 10^{-8} M provide essentially no effect
on cell proliferation, as also shown in Figure 11.

15   It is well known that the compound TTNPB activates
members of the RAR subfamily and has no significant activity for
members of the RXR subfamily. The compound TTNPB is shown below,
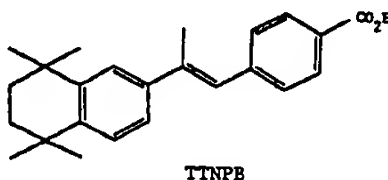and its activity is shown in Table 5.

TTNPB

TABLE 5

	Potency (nM)	Efficacy
TTNPB		
RXRα	>10,000	<5%
RXRβ	>10,000	<5%
RXRγ	>10,000	<5%
RARα	52	30
RARβ	4	40
RARγ	0.4	50

The effect of TTNPB on cell proliferation is shown in Figure 11. The IC_{50} value of TTNPB is about 5×10^{-11} M, and a concentration of less than 10^{-11} M produces essentially no effect on cell proliferation.

However, it has been found that when 3-methyl-TTNEB and TTNPB are present together, each at a concentration where the compound alone produces substantially no anti-proliferative effect, the combination of the two compounds effectively blocks cell proliferation. The combination of the two compounds appears to produce a greater than additive, or synergistic, effect.

For example, as shown in Figure 12, the presence of TTNPB at a concentration of 10^{-11} M produces a 9% inhibition on thymidine incorporation. However, combining it with 3-methyl-TTNEB at a concentration of 10^{-8} M (which results in no effect on cell proliferation) produces a greatly enhanced inhibitory effect of 49%. Likewise, it has also been found that the inhibitory effect of 3-methyl-TTNEB is greatly increased by the presence of TTNPB at a concentration which alone produces no effect.

Since it is well-known that toxic side effects of compounds such as TTNPB are concentration-dependent, the synergistic effect resulting from combining such RAR-specific compounds with RXR-specific compounds can be expected to permit

lower dosages that are efficacious and to therefore reduce toxic side effects. For example, in cancer chemotherapy, use of two such compounds, in combination, at relatively low doses can be expected to produce the desired beneficial effect, while minimizing

5   undesired side effects which result at higher doses of the compounds.

In vitro studies utilizing the co-transfection assay have also shown this same synergistic effect. For example, utilizing the co-transfection assay described previously and employing RAR-α

10   and RXR-α and a reporter consisting of the ApoA1 response element "A" site in the context of TKLUC (Ladias & Karathanasis, *Science* 251:561-65 (1991), transfections were performed in HEPG2 cells.   In this study, 100 ng of the designated receptor were used and RSVCAT was used as a carrier to keep the amount of RSV promoter constant.

15   All compounds were added at a final concentration of 10^{-7} M.   The RXR specific compound 3-methyl-TTNEB (Table 2, above) and the RAR specific compound TTNPB (Table 5, above) were utilized. As shown below in Table 6, the relative normalized response observed utilizing the co-transfection assay also demonstrated a synergistic

20   effect when a combination of the two compounds was utilized, compared to the response achieved utilizing the compounds individually.

TABLE 6

Compound	Reporter Activity (Fold Induction)
3-methyl-TTNEB	5
TTNPB	32
3-methyl-TTNEB + TTNPB	75

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As will be discernable to those skilled in the art from the foregoing discussions, the biological response of an RAR selective compound at a given concentration can be synergistically enhanced by combining the compound with an RXR selective compound.

5   Similarly, the biological response of an RXR selective compound can be enhanced by combining the compound with an RAR selective compound. Thus, it becomes possible to achieve a desirable biological response, using a combination of RAR and RXR selective compounds, at lower concentrations than would be the case using the

10   compounds alone. Among the advantages provided by such combinations of RAR and RXR selective compounds are desirable therapeutic effects with fewer side effects. In addition, novel effects that are not obtainable with either agent alone may be achieved by combinations of RAR and RXR selective compounds.

15   It has been further demonstrated that RXR-specific compounds also synergistically enhance the response of other hormonal systems. Specifically, peroxisome proliferator-activated receptor (PPAR) is a member of the intracellular receptor super family that plays a role in the modulation of lipid homeostasis.

20   PPAR has been shown to be achemotivated by amphipathic carboxylates, such as clofibric acid, and gemfibrizol. These agents, called peroxisome proliferators, have been used in man as hypolipidemic agents. The addition of 9-cis-retinoic acid (a retinoid ligand which activates both RAR and RXR receptors) and

25   clofibric acid to HepG2 cells transfected with RXRα and PPAR expression plasmids, results in the activation of receptor gene which was greater than the sum of the activation with each ligand separately. (Kliewer *et al., Nature* 358:771 (1992)). Similarly, when the above two receptors were co-transfected into HepG2 cells, the

30   addition of both an RXR-specific ligand (3-methyl-TTNEB) and

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clofibric acid was found to produce a greater than additive response as determined by activation of a target reporter gene, as shown below in Table 7.

TABLE 7

5

Compound	Normalized Response (%)
clofibric Acid	100
3-methyl-TTNEB	90
clofibric acid + 3-methyl-TTNEB	425

10   A similar synergistic effect was observed with RXR and RXR-specific ligands and the Vitamin D receptor (VDR) and its cognate ligands. When RXRβ and VD receptors were co-transfected into CV-1 cells containing a hormone response element, the addition of RXR selective 3-methyl-TTNCB and 1,25-dihydroxy-vitamin D (1,25-

15 D) produced a greater than additive response than was observed for each of the individual ligands, as shown below in Table 8.

TABLE 8

Compound	Normalized Response (%)
1,25-D	100
3-Methyl-TTNCB	13
1,25-D + 3-methyl-TTNCB	190

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    As shown, the above results indicate that each pair of receptors (RXRα/PPAR and RXRβ/VDR, respectively), in the presence of ligands known to specifically activate their respective

25 receptors, are capable of producing a synergistic response. The results indicate that the response of a single agent can be enhanced by the combination of the two agents, or that comparable biological or therapeutic responses can be achieved by use of lower doses of such agents in combination.

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The observation that RXR-specific ligands are able to act synergistically with RAR ligands, PPAR ligands, and Vitamin D ligands indicates that RXR-specific ligands have usefulness not only as single therapeutic agents but also in combination therapy

5   to obtain enhanced biological or therapeutic response by the addition of the RXR-specific ligand. Such combination therapy also may provide an added benefit of decreasing the side effects associated with the primary agent by employing lower doses of that agent. For example, use of Vitamin D or a related Vitamin D

10   receptor ligand in conjunction with an RXR selective compound for the treatment of a variety of disorders including skin diseases (acne, psoriasis), hyperproliferative disorders (benign and malignant cancers) and disorders of calcium homeostasis may decrease the adverse side effects associated with Vitamin D therapy

15   alone.

As a further example, the RXR-specific compounds of this invention have been demonstrated *in vitro* to act synergistically with compounds which affect cellular proliferation, such as Interferon. Specifically, the growth properties of two human tumor cell lines

20   (ME180, a squamous cell carcinoma, and RPMI18226, a multiple myeloma) were monitored in the presence of the compound 3-methyl-TTNEB alone and in combination with Interferonα2b, utilizing standard cell culture procedures. The effects on growth of these cells were monitored by evaluation of cell number, and also by

25   evaluation of growth in semi-solid medium for the RPMI18226 cell line. Both 3-methyl-TTNEB and Interferonα2b were found to inhibit cell growth in a concentration-dependent manner, and each alone to produce a significant depression in cell proliferation. In addition, when the cells were treated with both compounds, an

30   additive or a greater than additive effect on the depression in

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cell proliferation was observed. Treatment with other chemo-
therapeutic agents including anti-proliferative agents and/or cell-
cycle modulators (*e.g.*, methotrexate, fluorouracil (5-FU), ARA-C,
etc.) in combination with RXR-specific compounds would be expected

5   to produce similar results. The enhanced anti-proliferative effect
can be expected to permit lower therapeutic doses in treatment of
proliferative disorders, such as squamous cell and other
carcinomas. In addition, combination therapy could allow the use
of lower doses of these compounds to achieve a comparable

10 beneficial effect along with fewer side effects/toxic effects,
thereby enhancing the therapeutic index of the therapy. The
therapeutic index is defined as the ratio of efficacy to toxicity
of a compound.

Since RXR is known to form heterodimers with various

15 members of the intracellular receptor super family, it can be
expected that the synergistic response observed with use of RXR-
selective ligands may be achieved with other receptors with which
heterodimers are formed. These include PPARs, RARs, Vitamin D,
thyroid hormone receptors, HNF4, the COUP family of receptors, as

20 referenced above, and other as yet unidentified members of the
intracellular super family of receptors.

As will be further discernible to those skilled in the
art, the compounds disclosed above can be readily utilized in
pharmacological applications where selective retinoid receptor

25 activity is desired, and where it is desired to minimize cross
reactivities with other related intracellular receptors. *In vivo*
applications of the invention include administration of the
disclosed compounds to mammalian subjects, and in particular to
humans.

The compounds of the present invention are small molecules which are relatively fat soluble or lipophilic and enter the cell by passive diffusion across the plasma membrane. Consequently, these ligands are well suited for administration

5   orally and by injection, as well as topically. Upon administration, these ligands can selectively activate retinoid X receptors, and thereby selectively modulate processes mediated by these receptors.

The pharmaceutical compositions of this invention are

10   prepared in conventional dosage unit forms by incorporating an active compound of the invention, or a mixture of such compounds, with a nontoxic pharmaceutical carrier according to accepted procedures in a nontoxic amount sufficient to produce the desired pharmacodynamic activity in a mammalian and in particular a human

15   subject. Preferably, the composition contains the active ingredient in an active, but nontoxic, amount selected from about 5 mg to about 500 mg of active ingredient per dosage unit. This quantity depends on the specific biological activity desired and the condition of the patient.

20   The pharmaceutical carrier or vehicle employed may be, for example, a solid or liquid. A variety of pharmaceutical forms can be employed. Thus, when using a solid carrier, the preparation can be plain milled, micronized in oil, tableted, placed in a hard gelatin or enteric-coated capsule in micronized powder or pellet

25   form, or in the form of a troche, lozenge, or suppository. When using a liquid carrier, the preparation can be in the form of a liquid, such as an ampule, or as an aqueous or nonaqueous liquid suspension. For topical administration, the active ingredient may be formulated using bland, moisturizing bases, such as ointments or

30   creams. Examples of suitable ointment bases are petrolatum, petrolatum plus volatile silicones, lanolin, and water in oil

emulsions such as Eucerin (Beiersdorf). Examples of suitable cream bases are Nivea Cream (Beiersdorf), cold cream (USP), Purpose Cream (Johnson & Johnson) hydrophilic ointment (USP), and Lubriderm (Warner-Lambert).

5   The following examples provide illustrative pharmacological composition formulations:

Example 59

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
3-methyl-TTNCB	140
Starch, dried	100
Magnesium stearate	10
Total	250 mg

The above ingredients are mixed and filled into hard gelatin capsules in 250 mg quantities.

Example 60

A tablet is prepared using the ingredients below:

	Quantity (mg/tablet)
3-methyl-TTNCB	140
Cellulose, microcrystalline	200
Silicon dioxide, fumed	10
Stearic acid	10
Total	360 mg

The components are blended and compressed to form tablets each weighing 360 mg.

Example 61

Tablets, each containing 60 mg of active ingredient, are made as follows:

	Quantity (mg/tablet)
3-methyl-TTNCB	60
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (PVP) (as 10% solution in water)	4
Sodium carboxymethyl starch (SCMS)	4.5
Magnesium stearate	0.5
Talc	1.0
Total	150 mg

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of PVP is mixed with the resultant powders, which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The SCMS, magnesium stearate, and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Example 62

Suppositories, each containing 225 mg of active ingredient, may be made as follows:

3-methyl-TTNCB	225 mg
Saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously

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melted using the minimum heat necessary.   The mixture is then
poured into a suppository mold of normal 2g capacity and allowed to
cool.

Example 63

5   An intravenous formulation may be prepared as follows:

3-methyl-TTNCB	100 mg
Isotonic saline	1,000 ml
Glycerol	100 ml

The compound is dissolved in the glycerol and then the
10   solution is slowly diluted with isotonic saline.   The solution of
the above ingredients is then administered intravenously at a rate
of 1 ml per minute to a patient.

The compounds of this invention also have utility when
labeled as ligands for use in assays to determine the presence of
15   RXRs.   They are particularly useful due to their ability to
selectively bond to members of the RXR subfamily and can therefore
be used to determine the presence of RXR isoforms in the presence
of other related receptors.

Due to the selective specificity of the compounds of this
20   invention for retinoid X receptors, these compounds can also be
used to purify samples of retinoid X receptors *in vitro*.   Such
purification can be carried out by mixing samples containing
retinoid X receptors with one of more of the bicyclic derivative
compounds disclosed so that the compound (ligand) binds to the
25   receptor, and then separating out the bound ligand/receptor
combination by separation techniques which are known to those of
skill in the art.   These techniques include column separation,

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filtration, centrifugation, tagging and physical separation, and antibody complexing, among others.

   While the preferred embodiments have been described and illustrated, various substitutions and modifications may be made
5 thereto without departing from the scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustration and not limitation.